# AIA-360 Assay Specifications
# ST HOMOCYSTEINE Test Code 097

| | Item | Data |
|---|---|---|
| | Calib. Req | SHOW |
| 2 | Cal 1 | 0.0 |
| 3 | Cal 2 | 2.0 (example) |
| 4 | Cal 3 | 4.1 (example) |
| 5 | Cal 4 | 8.0 (example) |
| 6 | Cal 5 | 15.0 (example) |
| 7 | Cal 6 | 55.0 (example) |
| 8 | Cal Lot L | |
| 9 | Cal Lot R | |
| 10 | Unit | μmol/L |
| 11 | Smpl. Vol | 10 |
| 12 | Dil. Vol | 140 |
| 13 | Assay L | 0.5 |
| 14 | Assay H | 50.0 |
| 15 | Ref. L | 0.0 |
| 16 | Ref. H | 0.0 |
| 17 | Decimal | 1 |

**Visible only in Test Mode**

| | | |
|---|---|---|
| 18 | Test Name | #HCY |
| 19 | Calib. No | 6 |
| 20 | Calib. Mul | 3 |
| 21 | Calib. Equ | 6 |
| 22 | Calib. CV | 90 |
| 23 | Assay Prtl | 1 |
| 24 | Factor1 A | 1 |
| 25 | Factor1 B | 0 |
| 26 | Factor2 A | 1 |
| 27 | Factor2 B | 0 |
| 28 | V. Conc | 0.00 |
| 29 | G Origin | 0.00 |

# AIA-900 Assay Specifications
# ST HOMOCYSTEINE Test Code 097

| No. | Item | Data |
|---|---|---|
| 1 | Code | 097 |
| 2 | ACT | 1 |
| 3 | Analyte | #HCY |
| 4 | Lot | ***Enter current lot number |
| 5 | CAL 1 | 0.0 |
| 6 | CAL 2 | 2.0 (example) |
| 7 | CAL 3 | 4.1 (example) |
| 8 | CAL 4 | 8.0 (example) |
| 9 | CAL 5 | 15.0 (example) |
| 10 | CAL 6 | 55.0 (example) |
| 11 | Cal lot L | |
| 12 | Cal lot R | |
| 13 | Unit | μmol/L |
| 14 | Decimal | 1 |
| 15 | Assay Low | 0.5 |
| 16 | Assay High | 50.0 |
| 17 | Reference Low | |
| 18 | Reference High | |
| 19 | Reschedule Low | 0.5 |
| 20 | Reschedule High | 50.0 |
| 21 | Factor A | 1 |
| 22 | Factor B | 0 |
| 23 | Sample Volume | 10 |
| 24 | Diluent Volume | 140 |
| 25 | 2 Step reagent dispensing volume | |
| 26 | Calibration code | 97 |
| 27 | CAL. No. | 6 |
| 28 | CAL. MUL. | 3 |
| 29 | CAL. EQU. | 6 |
| 30 | CAL. CV | 90 |
| 31 | DIL. SP1 | 1 |
| 32 | DIL. SP2 | 1 |
| 33 | DIL. CAL. (Calculation of dil ratio of conc.) | 1 |
| 34 | DIL. CNTL. | 1 |
| 35 | DIL. DO | 1 |
| 36 | DIL. AH. | 5 |
| 37 | DIL. CALC. | 1 |
| 38 | DIL. CODE | 97 |
| 39 | DIL. NAME | #HCY |
| 40 | DIL. PRTY | 3 |
| 41 | PRE. SPVOL (Vol. of pretreated sample) | 40 |
| 42 | PRE. 1VOL (Vol. of pretreatment sol-1) | 200 |
| 43 | PRE. 2 VOL (Vol. of pretreatment sol-2) | 0 |
| 44 | PRE. CODE (pretreated sol. code) | 97 |
| 45 | PRE. NAME (pretreated sol. name) | #HCY |
| 46 | Protocol | 1 |
| 47 | SYS. F_A | 1 |
| 48 | SYS. F_B | 0.00 |

# AIA-2000 Assay Specifications
# ST HOMOCYSTEINE Test Code 097

| No. | Item | Data |
|---|---|---|
| 1 | Unit | µmol/L |
| 2 | Decimal places | 1 |
| 3 | Reference low | |
| 4 | Reference high | |
| 5 | Reschedule low | 0.5 |
| 6 | Reschedule high | 50.0 |
| 7 | Assay range low | 0.5 |
| 8 | Assay range high | 50.0 |
| 9 | Specimen diluent code | 97 |
| 10 | Specimen diluent name | #HCY |
| 11 | Dilution factor for Sp. 1 | 1 |
| 12 | Dilution factor for Sp. 2 | 1 |
| 13 | Dilution factor for Control | 1 |
| 14 | Default multiplier for DO | 1 |
| 15 | Default multiplier for >H | 5 |
| 16 | Factor A | 1 |
| 17 | Factor B | 0 |
| 18 | Calibration code | 6 |
| 19 | Calibrator replicates | 3 |
| 20 | Calibrator lot | ***Enter current lot number |
| 21 | Calibrator Concentration | |
| 22 | Cal - 01 | 0.0 |
| 23 | Cal - 02 | 2.0 (example) |
| 24 | Cal - 03 | 4.1 (example) |
| 25 | Cal - 04 | 8.0 (example) |
| 26 | Cal - 05 | 15.0 (example) |
| 27 | Cal - 06 | 55.0 (example) |
| 28 | | |
| 29 | | |
| 30 | | |
| 31 | | |
| 32 | | |
| 33 | | |
| 34 | Dilution factor for calibrator | 1 |
| 35 | Calibration period | 90 |
| 36 | Incubation time (10/40) | 10 |
| 37 | Assay protocol | 1 |
| 38 | Specimen volume | 10 |
| 39 | Diluent volume | 140 |
| 40 | Conjugate volume | 0 |
| 41 | Diluted conjugate volume | 0 |
| 42 | Pretreatment | 1 |
| 43 | Pretreatment specimen volume | 40 |
| 44 | Pretreatment Reagent code | 97 |
| 45 | Pretreatment Reagent name | #HCY |
| 46 | Pretreatment Reagent 1 volume | 200 |
| 47 | Pretreatment Reagent 2 volume | 0 |
| 48 | System Factor | 1 |
| 49 | Calibration Code Check | 097 |
| 50 | Virtual Concentration | 0.0 |
| 51 | Graph Origin | 0.0 |
| 52 | Calculation with Dilution Factor | Yes |

# HOMOCYSTEINE
# ST AIA-PACK HOMOCYSTEINE

## Name and Intended Use

ST AIA-PACK Homocysteine (HCY) is designed for IN VITRO DIAGNOSTIC USE ONLY for the quantitative measurement of homocysteine in human serum, heparinized plasma or EDTA plasma on Tosoh AIA System Analyzers. Homocysteine measurements are used in the diagnosis and treatment of hyperhomocysteinemia or homocysteinuria.

## Summary and Explanation of Test

Homocysteine (HCY) is a thiol-containing amino acid produced in the degradation of methionine. Homocysteine exported into plasma circulates mostly in its oxidized form, bound to plasma proteins (1 - 4). Smaller amounts of reduced and disulfide forms are also present. Total homocysteine is the sum of all homocysteine forms present in blood.

The metabolism of homocysteine is regulated by three enzymatic pathways and the pathways need vitamins (folic acid, vitamin B6 and vitamin B12) as cofactors (2, 4). Genetic disorders of enzymes involved in homocysteine metabolism results in homocystinuria. Deficiency of vitamins is a cause of elevated homocysteine levels in plasma or serum (5 - 8).

## Principle of the Assay

The ST AIA-PACK Homocysteine is a competitive enzyme immunoassay which, after sample pretreatment, is performed entirely in the ST AIA-PACK Homocysteine test cups.

Oxidized homocysteine is reduced by tris (2-carboxyethyl) phosphine (TCEP) to the free form and converted to S-adenosyl-L-homocysteine (SAH) by the SAH hydrolase and excess adenosine prior to the immunoassay. SAH present in the pretreated sample competes with immobilized SAH on magnetic beads for binding sites of the enzyme-labeled anti-SAH mouse monoclonal antibody. The magnetic beads are washed to remove unbound anti-SAH mouse monoclonal antibody and are then incubated with a fluorogenic substrate, 4-methylumbelliferyl phosphate (4MUP). The rate of fluorescence produced by the enzyme reaction indicates the amount of enzyme-labeled anti-SAH mouse monoclonal antibody. The amount of antibody that binds to the beads is inversely proportional to the homocysteine concentration in the test sample. A standard curve is constructed, and unknown sample concentrations are calculated using this curve.

WARNING: Specimens from patients who are on drug therapy involving S-adenosyl-methionine may show falsely elevated levels of homocysteine. Specimens from patients taking methotrexate, carbamazepine, phenytoin, nitrous oxide or 6-azauridine triacetate may have elevated levels of homocysteine due to their effect on the metabolic pathway[15].

## Material Provided (ST AIA-PACK Homocysteine, Cat. No. 0025226)

5 trays x 20 test cups (ST AIA-PACK Homocysteine Test Cup)

The following materials are required to perform homocysteine analysis using the ST AIA-PACK Homocysteine (Cat. No. 0025226) on the Tosoh AIA System Analyzers. The following are available separately from Tosoh.

| **Materials** | | | | **Cat. No.** |
|---|---|---|---|---|
| AIA-2000 ST | | | | 0022100 |
| AIA-2000 LA | | | | 0022101 |
| AIA-900 | | | | 0022930 |
| AIA-900 9tray Sorter | | | | 0022931 |
| AIA-900 19tray Sorter | | | | 0022932 |
| AIA-360 | | | | 0019945 |
| AIA-PACK SUBSTRATE SET II | | | | 0020968 |
| AIA-PACK SUBSTRATE REAGENT II / AIA-PACK SUBSTRATE RECONSTITUENT II | | | | |
| ST AIA-PACK Homocysteine Calibrator Set | | | | 0025326 |
| ST AIA-PACK Homocysteine CALIBRATOR (1) | 0 | μmol/L | | |
| ST AIA-PACK Homocysteine CALIBRATOR (2) | 2 | μmol/L | (approx.) | |
| ST AIA-PACK Homocysteine CALIBRATOR (3) | 4 | μmol/L | (approx.) | |
| ST AIA-PACK Homocysteine CALIBRATOR (4) | 8 | μmol/L | (approx.) | |
| ST AIA-PACK Homocysteine CALIBRATOR (5) | 15 | μmol/L | (approx.) | |
| ST AIA-PACK Homocysteine CALIBRATOR (6) | 55 | μmol/L | (approx.) | |
| AIA-PACK Homocysteine Control Set | | | | 0025426 |
| AIA-PACK Homocysteine CONTROL LEVEL 1 | 12 | μmol/L | (approx.) | |
| AIA-PACK Homocysteine CONTROL LEVEL 2 | 25 | μmol/L | (approx.) | |
| ST AIA-PACK Homocysteine SAMPLE DILUTING SOLUTION | | | | 0025526 |
| ST AIA-PACK Homocysteine PRETREATMENT SET | | | | 0025726 |
| ST AIA-PACK Homocysteine PRETREATMENT-1 | | | | |
| ST AIA-PACK Homocysteine PRETREATMENT-2 | | | | |
| AIA-PACK WASH CONCENTRATE | | | | 0020955 |
| AIA-PACK DILUENT CONCENTRATE | | | | 0020956 |
| SAMPLE CUPS | | | | 0018581 |
| AIA-PACK DETECTOR STANDARDIZATION TEST CUP | | | | 0020970 |
| AIA-PACK SAMPLE TREATMENT CUP | | | | 0020971 |
| Additional Requirements for the AIA-900 and AIA-2000: | | | | |
| PIPETTE TIPS (1000/PKG) | | | | 019215 |
| TIP RACK (EMPTY) | | | | 019216 |
| PRELOADED PIPETTE TIPS (96 TIPS X 50 RACKS) | | | | 996010 |
| PRELOADED PIPETTE TIPS (96 TIPS X 50 RACKS) | | | | 996005 |

Only materials obtained from Tosoh should be used. Materials obtained elsewhere should not be substituted since assay performance is characterized based strictly on Tosoh materials.

## Warnings and Precautions

- The ST AIA-PACK Homocysteine is intended for in vitro diagnostic use only.
- Rx only. US Federal law restricts this device to sale by or on the order of a licensed healthcare practitioner.
- Inspect the packaging and the exterior of the aluminum pouch for any sign of damage before use. If any damages are visible, contact your local Tosoh sales representative.
- Test cups from different lots or different assays should not be mixed within a tray.
- ST AIA-PACK Homocysteine, ST AIA-PACK Homocysteine CALIBRATOR SET, ST AIA-PACK Homocysteine SAMPLE DILUTING SOLUTION and ST AIA-PACK Homocysteine PRETREATMENT-2 contain sodium azide, which may react with lead or copper plumbing to form potentially explosive metal azides. When disposing of such reagents, always flush with large volumes of water to prevent azide build-up.
- The material derived from human origin is not used in the preparation of this product; however, since human specimens will be used for samples and other quality control products in the lab may be derived from human origin, please use standard laboratory safety procedures in handling all specimens and controls.
- Do not use beyond the expiration date.
- For safe waste disposal, it is recommended that each laboratory complies with established laboratory procedures and local, state, and federal regulations.
- After opening, the vial of ST AIA-PACK Homocysteine SAMPLE DILUTING SOLUTION should be kept tightly sealed with a clean rubber cap. Sealing with dirty material may cause deterioration of the reagent.
- The remaining sample diluting solution after use should not be mixed with another vial but be discarded to avoid contamination.
- The ST AIA-PACK Homocysteine PRETREATMENT SETs with different lot numbers should not be mixed.
- Do not pool the remaining pretreatment reagents after use.
- Serum, dust, metal, or microorganism contamination may cause degradation of reconstituted substrate solution. Store in a clean environment, away from direct sunlight and ultraviolet light.

## Storage and Stability

All unopened materials are stable until the expiration date on the label when stored at the specified temperature.

| Materials | Cat. No. |
|---|---|
| **Refrigerator Temperature (2 - 8 °C):** | |
| ST AIA-PACK Homocysteine | 0025226 |
| ST AIA-PACK Homocysteine Calibrator Set | 0025326 |
| ST AIA-PACK Homocysteine Sample Diluting Solution | 0025526 |
| AIA-PACK Homocysteine Control Set | 0025426 |
| ST AIA-PACK Homocysteine Pretreatment Set | 0025726 |
| AIA-PACK SUBSTRATE SET II | 0020968 |
| AIA-PACK WASH CONCENTRATE | 0020955 |
| AIA-PACK DILUENT CONCENTRATE | 0020956 |
| **Room Temperature (18 - 25 °C):** | |
| AIA-PACK DETECTOR STANDARDIZATION TEST CUP | 0020970 |
| AIA-PACK SAMPLE TREATMENT CUP | 0020971 |

The shelf life of the ST AIA-PACK Homocysteine test cups, ST AIA-PACK Homocysteine Calibrator Set, ST AIA-PACK Homocysteine Sample Diluting Solution, ST AIA-PACK Homocysteine Pretreatment Set and the AIA PACK Homocysteine Control Set is 12 months from the date of manufacture when stored at 2 - 8 °C.

The in-use stability of the ST AIA-PACK Homocysteine test cups is 40 hours at a room temperature of 18 - 25 °C. When stored at 2 - 8 °C, the test cups can be used for up to 30 days. The in-use stability of the ST AIA-PACK Homocysteine Calibrator Set is 1 day when stored at 2 - 8 °C. The in-use stability of the ST AIA-PACK Homocysteine Sample Diluting Solution is 9 days provided: 1) it is used for automatic dilutions, 2) it is at 18 - 25 °C for only 8 hours per day, and 3) the vials are closed and kept refrigerated immediately after use. In-use stability is 90 days provided: 1) it is used for manual dilutions ONLY, and 2) the vials are closed and refrigerated immediately after use. The in-use stability of the ST AIA-PACK Homocysteine Pretreatment reagent is stable at 18 - 25 °C for 20 hours. The in-use stability is stable at 2 - 8 °C for 1 day provided: 1) it is used for manual pretreatment ONLY, and 2) the bottles are closed and refrigerated immediately after use. The in-use stability of the AIA-PACK Homocysteine CONTROL is 14 days at 2 - 8 °C. If stored at 18 - 25 °C the in-use stability is 1 day.
Reconstituted substrate solution is stable for 3 days at 18 - 25 °C or 30 days at 2 - 8 °C. Working diluent and wash solutions are stable for 30 days at room temperature (18 - 25 °C). Reagents should not be used if they appear cloudy or discolored.

## Specimen Collection and Handling

- Serum, heparinized plasma or EDTA plasma is required for the assay. Citrated plasma SHOULD NOT BE USED.
- To avoid increases in homocysteine concentration from synthesis by red blood cells, all specimens must be placed on ice immediately after collection. It is important to separate the plasma or serum from the cells after collection. Specimens not placed on ice immediately may exhibit a 10-50% increase in concentration (15).
- When using EDTA or heparinized plasma, a venous blood sample is collected aseptically with the designated additive. Centrifuge and separate plasma sample from the packed cells as soon as possible.
- When using serum, a venous blood sample is collected aseptically without additives. Store at 18 - 25 °C until a clot has formed (usually 15 - 45 minutes), then centrifuge to obtain the serum specimen for assay. After a complete clot is formed, centrifuge and separate serum sample from the packed cells as soon as possible. Serum may clot more slowly and the volume of serum may be reduced as a result of being on ice.
- Inadequate centrifugation or the presence of fibrin or particulate matter in the sample may cause an erroneous result.
- Samples containing inhibitors of alkaline phosphatase may cause erroneous results.
- Inspect all samples for air bubbles and foaming. Remove any air bubbles prior to assay.
- Plasma or Serum samples may be stored at 2 - 8 °C for up to 24 hours prior to analysis. If the analysis cannot be done within 24 hours, the sample should be stored frozen at -20 °C or below for up to 60 days.
- Repeated freeze-thaw cycles should be avoided. Turbid samples or samples containing particulate matter should be centrifuged prior to testing. Prior to assay, slowly bring frozen samples to 18 - 25 °C and mix gently.
- The sample volume required for pretreatment is 40 µL. 10 µL of the pretreated

sample is used for the assay.

# Procedure

For the AIA-900, AIA-2000 and AIA-360, please refer to their Operator's Manual for detailed instructions.

## 1) Reagent Preparation

### 1a) Substrate Solution

Bring all reagents to room temperature (18 - 25 °C) before preparing the working reagent. Add the entire contents of the Substrate Reconstituent (100 mL) to the lyophilized Substrate and mix thoroughly to dissolve the solid material.

### 1b) Wash Solution

Add the entire contents of the Wash Concentrate (100 mL) to approximately 2.0 L of CAP Class I water or the clinical laboratory reagent water, mix well, and adjust the final volume to 2.5 L.

### 1c) Diluent

Add the entire contents of the Diluent Concentrate (100 mL) to approximately 4.0 L of CAP Class I water or the clinical laboratory reagent water, mix well, and adjust the final volume to 5.0 L.

### 1d) Pretreatment Reagent

Bring all pretreatment reagents to 18 - 25 °C. Using a volumetric pipette or a calibrated adjustable pipette, reconstitute the lyophilized ST AIA-PACK Homocysteine PRETREATMENT-1 accurately with 5 mL of ST AIA-PACK Homocysteine PRETREATMENT-2. Allow the lyophilized material to fully dissolve. The reconstituted pretreatment-1 should be used within 1 day.

## II. Pretreatment Procedure

All samples (plasma, serum, controls, and calibrators) require pretreatment prior to assaying on the Tosoh AIA System Analyzers. Pretreatment on the AIA-900 and AIA-2000 is performed automatically using the parameters defined in the test file. On the AIA-360, manual pretreatment is necessary. Follow the instructions for the automatic / manual pretreatment procedure below. The pretreated samples should be assayed within 2 hours after completion of pretreatment procedure. Refer to the TOSOH AIA System Operator's Manual for additional instructions regarding pretreatment procedure.

A. Automatic Pretreatment Procedure

1. Bring all pretreatment reagents and samples to 18 - 25 °C
2. Using a volumetric pipette or a calibrated adjustable pipette, reconstitute the lyophilized ST AIA-PACK Homocysteine PRETREATMENT-1 accurately with 5 mL of ST AIA-PACK Homocysteine PRETREATMENT-2. Allow the lyophilized material to fully dissolve.
3. Place the reconstituted pretreatment-1 in the designated position of the reagent

cassette as defined under REAGENT REGISTRATION. On the AIA-900, place the vial in the cassette and register it by the registration screen of the analyzers. On the AIA-2000, place the vial in the cassette with the barcode label facing the front.

4. Place the appropriate amount of sample cups / primary tubes, AIA-PACK SAMPLE TREATMENT CUPs and ST AIA-PACK Homocysteine test cups on the instrument.

B. Manual Pretreatment Procedure

1. Bring all pretreatment reagents and samples to 18 - 25 °C.
2. Using a volumetric pipette or a calibrated adjustable pipette, reconstitute the lyophilized ST AIA-PACK Homocysteine PRETREATMENT-1 accurately with 5 mL of ST AIA-PACK Homocysteine PRETREATMENT-2. Allow the lyophilized material to fully dissolve.
3. Pipette 200 μL of the reconstituted pretreatment-1 into a disposable tube.
4. Pipette 40 μL of a sample (plasma, serum, control, or calibrator) into each tube.
5. Cover the tubes and mix well using a vortex mixer.
6. Incubate the sample for 30 minutes at 18 - 25 °C.
7. Transfer all of the pretreated samples into sample cups.
8. Place the sample cups on the instrument.
9. The pretreated samples are ready for the assay with the ST AIA-PACK Homocysteine. The pretreated samples should be assayed within 2 hours after completion of pretreatment. The pretreated samples should not be stored as frozen.

## III. Calibration Procedure

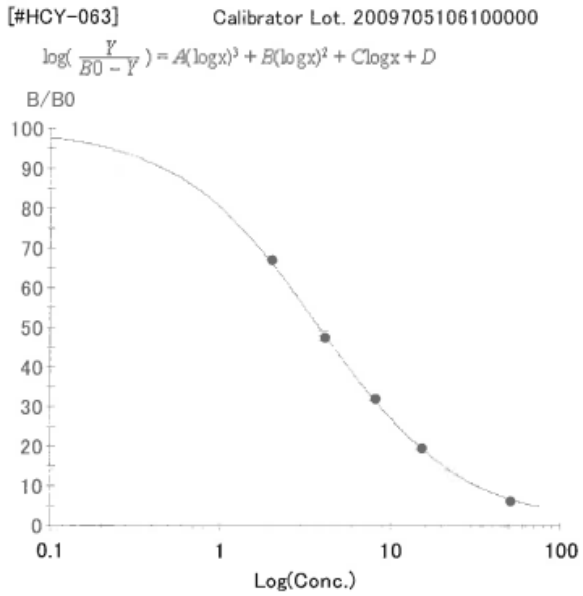
### A. Calibration Curve

The calibrators for use with the ST AIA-PACK Homocysteine are prepared gravimetrically and are referred to NIST (National Institute of Standards & Technology) Standard Reference Material 1955.

The calibration curve for the ST AIA-PACK Homocysteine is stable for up to 90 days. Calibration stability is monitored by quality control performance and is dependent on proper reagent handling and Tosoh AIA System maintenance according to the manufacturer's instructions.

Recalibration may be necessary more frequently if controls are out of the established range for this assay or when certain service procedures are performed (e.g. temperature adjustment, sampling mechanism changes, maintenance of the wash probe or detector lamp adjustment or change).

A sample calibration curve from the AIA-2000 follows and shows the algorithm used for calculating results.




## B. Calibration Procedure

Refer to the appropriate Tosoh AIA System Operator's Manual for the procedural instructions.

1. Verify that both the calibrator lot and concentration numbers have been correctly entered into the software.
2. The ST AIA-PACK Homocysteine CALIBRATOR SET is provided ready for use.
3. Tosoh recommends that all calibrators be run in triplicate.

## C. Calibration Acceptability Criteria

1. Since there is an inverse relationship between concentration and rate, the rate should decrease as the concentration increases.
2. The replicate values should be within a 10% range.

## D. Calibration Review and Acceptance

1. Review the calibration curve carefully, using the criteria listed above.
2. Edit the calibration if necessary, then accept the calibration.
   For further information regarding calibration, consult the Tosoh AIA System Operator's Manual.

## IV. Quality Control Procedure

### A. Commercially Available Controls

Commercially available controls should be run at least once per day. It is recommended that at least two levels of controls, normal and abnormal, be used. Laboratory policy for this particular assay designates the following:

Control Material:_____
Frequency: _____

Lot number of control material, acceptable limits, and corrective action to be taken if controls do not meet laboratory criteria will be found in a separate quality control document maintained by the laboratory.

### B. Quality Control Procedure

1. Assay quality control specimens as instructed in the specific Operator's Manual for your analyzer. In addition, refer to the Tosoh AIA System Operator's Manual for detailed instructions on defining and editing the files.
2. Quality control material to be run with this assay is defined by individual laboratory policy.

## V. Specimen Processing

### A. Preparation

All patient specimens require pretreatment prior to analysis. Following the specific instructions in the Operator's Manual for the analyzer, place either non-pretreated samples (on the AIA-900 and AIA-2000) or pretreated samples (on the AIA-360) in the appropriate position on Tosoh AIA System Analyzers. For the AIA-360, the sample manually pretreated in a tube must be transferred to a sample cup and placed on the analyzer. For the AIA-900 and AIA-2000, barcoded primary tubes as well as sample cups can be run.

For further information regarding sample preparation and instrument operation, refer to the SPECIMEN COLLECTION AND HANDLING section in this insert sheet and consult the specific Tosoh AIA System Operator's Manuals, respectively.

### B. Assay Procedure

1. Ensure a sufficient quantity of ST AIA-PACK Homocysteine test cups and The Pretreatment Reagent for the number of samples to be run.
2. Load patient samples as instructed in the Operator's Manual and proceed with analysis. Note: The AIA-900 and AIA-2000 will require AIA-PACK SAMPLE TREATMENT CUPs.

## Procedural Notes

1. Lyophilized Substrate must be completely dissolved.
2. Ligand assays performed by the Tosoh AIA System Analyzer require that the laboratory use water designated by the CAP Class I or by the clinical laboratory reagent water. Water should be tested at least once per month and should be free of particulate matter including bacteria. The pH of the water should also be routinely tested. For further information, consult the CLSI document GP40-A4-AMD, Preparation and Testing of Reagent Water in the Clinical Laboratory; Approved Guideline - Fourth Edition.
3. If a specimen homocysteine concentration is found to be greater than the upper limit of the assay range, 50.0 μmol/L, the specimen should be diluted with the ST AIA-PACK Homocysteine SAMPLE DILUTING SOLUTION and reassayed according to the Assay Procedure. The recommended dilution for specimens containing greater than 50.0 μmol/L is 5 fold dilution. It is desirable to dilute the specimen so that the diluted specimen reads between 0.50 and 50.0 μmol/L. Dilution is performed automatically using the dilution factor defined in the test file. For further information on the dilution of specimens, refer to the Tosoh AIA System Operator's Manual.
4. The Tosoh AIA System Analyzer can store two different calibration curves for each analyte at one time. Therefore, up to two different lots of ST AIA-PACK Homocysteine test cups can be used during the same run.
5. If the assay specifications for this test are not ready in the system software, the specifications must be entered under test code 097.

## Calculation of Results

The Tosoh AIA System Analyzers perform all sample and reagent handling operations automatically except the pretreatment procedure for the AIA-360 which should be done manually. The Tosoh AIA System Analyzers read the rate of fluorescence produced by the reaction and automatically convert the rate to homocysteine concentration in μmol/L.

For samples requiring dilution, AIA-900 and AIA-2000 will automatically perform dilutions and calculate results if the dilution factors are entered into the software. For detailed information regarding programming dilutions, consult the appropriate Tosoh AIA System Operator's Manual.

## Evaluation of Results

### Quality Control

In order to monitor and evaluate the precision of the analytical performance, it is recommended that commercially available control samples be assayed daily.

The minimum recommendations for the frequency of running internal control material are:

- After calibration, two levels of controls are run in order to accept the calibration curve.
- The two levels of controls are also repeated after calibration when certain service procedures are performed (e.g. temperature adjustment, sampling mechanism changes, maintenance of the wash probe or detector lamp adjustment or change).
- After daily maintenance, two levels of the control should be run in order to verify the overall performance of the Tosoh AIA System Analyzers.

If one or more control sample value(s) is out of the acceptable range, it will be necessary to investigate the validity of the calibration curve before reporting patient results.

Standard laboratory procedures should be followed in accordance with the regulatory agency under which the laboratory operates.

## Limitations of the Procedure

WARNING: Specimens from patients who are on drug therapy involving S-adenosyl-methionine may show falsely elevated levels of homocysteine. Specimens from patients taking methotrexate, carbamazepine, phenytoin, nitrous oxide or 6-azauridine triacetate may have elevated levels of homocysteine due to their effect on the metabolic pathway[15].

For diagnostic purposes, the results obtained from this assay should be used in conjunction with other data (e.g. symptoms, results of other tests, clinical impressions, therapy, etc.).

Using the ST AIA-PACK Homocysteine, the highest measurable concentration of homocysteine in specimens without dilution is 50.0 μmol/L, and the lowest measurable concentration in specimens is 0.50 μmol/L (assay sensitivity).

Although the approximate value of the highest calibrator is 55.0 μmol/L, the exact concentration may be slightly different. The assay specification, ASSAY RANGE HIGH, should be defined as the upper limit of the assay range, 50.0 μmol/L.

Although hemolysis has insignificant effect on the assay, hemolyzed samples may indicate mistreatment of a specimen prior to assay and results should be interpreted with caution.

Lipemia has an insignificant effect on the assay except in the case of gross lipemia where spatial interference may occur.

Certain medications may interfere with assay performance. Specimens from patients taking medicines and/or medical treatment may show erroneous results.[12-13] All results should be interpreted with respect to the clinical picture of the patient.

Samples from patients who had an injection of fluorescein, which is used in fluorescein fundus angiography, may cause falsely elevated results.

Specimens containing heterophilic antibodies may give falsely elevated or decreased homocysteine concentrations.

For a more complete understanding of the limitations of this procedure, please refer to the Specimen Collection and Handling, Warnings and Precautions, Storage and Stability, and Procedural Notes sections in this Technical Document.

## Expected Values

Each laboratory should determine a reference interval which corresponds to the characteristics of the population being tested. As with all diagnostic procedures, clinical results must be interpreted with regard to concomitant medications administered to the patient.

## Reference Ranges

The interval given here was determined in unaltered EDTA plasma samples from 130 apparently healthy individuals.

A reference range study was conducted based on guidance from Clinical and Laboratory Standards Institute (CLSI) Protocol C28-A2.

Number of Samples (n) 130
Reference Interval 6.6 - 17.8 μmol/L

The central 95% of the reference range was used to determine the reference interval.

## Performance Characteristics

The following performance characteristics were determined using the Tosoh AIA-2000 Automated Immunoassay Analyzer. All Tosoh AIA System Analyzers demonstrate equivalent performance.

### 1) Accuracy

#### 1a) Recovery:

**a.** Three EDTA plasma and three serum samples were spiked with three different levels of homocysteine and assayed before and after spiking.

| Sample | Initial Value (μmol/L) | HCY Added (μmol/L) | Expected Value (μmol/L) | Measured Value (μmol/L) | Percent Recovery (%) |
|---|---|---|---|---|---|
| Plasma A1 | 5.7 | 4.8 | 10.6 | 10.5 | 99.5 |
| | 5.7 | 9.7 | 15.4 | 15.8 | 102.7 |
| | 5.7 | 19.3 | 25.1 | 26.1 | 104.0 |
| Plasma B1 | 9.0 | 4.8 | 13.8 | 14.2 | 102.7 |
| | 9.0 | 9.7 | 18.7 | 18.3 | 98.0 |
| | 9.0 | 19.3 | 28.3 | 28.2 | 99.4 |
| Plasma C1 | 15.0 | 4.8 | 19.9 | 20.1 | 101.3 |
| | 15.0 | 9.7 | 24.7 | 24.0 | 97.3 |
| | 15.0 | 19.3 | 34.4 | 34.4 | 100.2 |
| Serum A1 | 4.5 | 4.7 | 9.2 | 9.3 | 101.0 |
| | 4.5 | 9.4 | 13.9 | 15.0 | 107.9 |
| | 4.5 | 18.7 | 23.3 | 23.0 | 98.7 |
| Serum B1 | 7.8 | 4.7 | 12.4 | 12.6 | 101.2 |
| | 7.8 | 9.4 | 17.1 | 16.9 | 98.4 |
| | 7.8 | 18.7 | 26.5 | 26.9 | 101.3 |
| Serum C1 | 12.7 | 4.7 | 17.4 | 17.8 | 102.0 |
| | 12.7 | 9.4 | 22.1 | 22.5 | 102.0 |
| | 12.7 | 18.7 | 31.5 | 32.6 | 103.6 |

### 1b) Dilution:

Three EDTA plasma and three serum samples containing high concentrations of homocysteine were serially diluted with the ST AIA-PACK Homocysteine SAMPLE DILUTING SOLUTION and assayed.

| Sample | Dilution Factor | Expected Value (μmol/L) | Measured Value (μmol/L) | Percent Recovery (%) |
|---|---|---|---|---|
| Plasma A2 | none | | 20.3 | |
| | 7.5/10 | 15.2 | 15.9 | 105.0 |
| | 5.0/10 | 10.1 | 10.7 | 105.3 |
| | 2.5/10 | 5.1 | 5.0 | 98.0 |
| | 1.0/10 | 2.0 | 2.1 | 105.0 |
| Plasma B2 | none | | 37.0 | |
| | 7.5/10 | 27.8 | 27.6 | 99.5 |
| | 5.0/10 | 18.5 | 17.6 | 95.1 |
| | 2.5/10 | 9.3 | 9.3 | 100.0 |
| | 1.0/10 | 3.7 | 3.8 | 102.7 |
| Plasma C2 | none | | 32.6 | |
| | 7.5/10 | 24.4 | 24.2 | 99.1 |
| | 5.0/10 | 16.3 | 15.8 | 96.7 |
| | 2.5/10 | 8.2 | 7.7 | 93.9 |
| | 1.0/10 | 3.3 | 3.2 | 97.0 |
| Serum A2 | none | | 19.6 | |
| | 7.5/10 | 14.7 | 14.8 | 100.5 |
| | 5.0/10 | 9.8 | 9.8 | 100.0 |
| | 2.5/10 | 4.9 | 4.9 | 100.0 |
| | 1.0/10 | 2.0 | 2.0 | 100.0 |
| Serum B2 | none | | 28.0 | |
| | 7.5/10 | 21.0 | 21.5 | 102.0 |
| | 5.0/10 | 14.0 | 14.4 | 102.7 |
| | 2.5/10 | 7.0 | 7.0 | 100.0 |
| | 1.0/10 | 2.8 | 2.5 | 89.3 |
| Serum C2 | none | | 30.4 | |
| | 7.5/10 | 22.8 | 22.8 | 99.9 |
| | 5.0/10 | 15.2 | 14.9 | 97.8 |
| | 2.5/10 | 7.6 | 7.2 | 94.7 |
| | 1.0/10 | 3.0 | 2.8 | 93.3 |

### 1c) Linearity:

The linearity for the ST AIA-PACK Homocysteine was determined, based on guidance from CLSI Protocol EP06-A. The linearity was measured on the AIA-2000 instrument and has been demonstrated to be linear from 0.5 to 50.0 μmol/L.

## 2) Precision

The precision for ST AIA-PACK Homocysteine was determined based on guidance from CLSI Protocol EP05-A2.

**2a)** Within run precision was determined using nine pooled samples (3 eacy of EDTA plasma, heparinized plasma and serum) in a total of 20 runs. Within each run, one set of duplicates per sample was assayed. The mean of each duplicate was used to obtain the pooled standard deviation (SD), which was then used to calculate the coefficient of variation (CV).

Intra-assay (within run) Precision

| Sample | Mean (μmol/L) | Standard Deviation (μmol/L) | Coefficient of Variation (%) |
|---|---|---|---|
| EDTA Plasma A3 | 7.4 | 0.2 | 3.3 |
| EDTA Plasma B3 | 17.4 | 0.7 | 3.9 |
| EDTA Plasma C3 | 44.0 | 1.4 | 3.1 |
| HEP Plasma A3 | 7.0 | 0.2 | 3.2 |
| HEP Plasma B3 | 14.4 | 0.5 | 3.4 |
| HEP Plasma C3 | 32.2 | 1.1 | 3.5 |
| Serum A3 | 5.6 | 0.2 | 4.3 |
| Serum B3 | 14.3 | 0.5 | 3.6 |
| Serum C3 | 40.9 | 1.3 | 3.2 |

**2b)** The total precision was determined by the duplicate assay of nine pooled samples (3 each of EDTA plasma, heparinized plasma and serum) in 20 separate runs. The means of each run were used to calculate the pooled standard deviation (SD) and coefficient of variation (CV).

Total Precision

| Sample | Mean (μmol/L) | Standard Deviation (μmol/L) | Coefficient of Variation (%) |
|---|---|---|---|
| EDTA Plasma A3 | 7.4 | 0.3 | 4.1 |
| EDTA Plasma B3 | 17.4 | 0.8 | 4.5 |
| EDTA Plasma C3 | 44.0 | 1.6 | 3.6 |
| HEP Plasma A3 | 7.0 | 0.3 | 4.4 |
| HEP Plasma B3 | 14.4 | 0.6 | 4.0 |
| HEP Plasma C3 | 32.2 | 1.6 | 4.9 |
| Serum A3 | 5.6 | 0.3 | 5.0 |
| Serum B3 | 14.3 | 0.7 | 4.7 |
| Serum C3 | 40.9 | 1.8 | 4.4 |

## Correlation

The methods comparison study was developed with the reference to the CLSI protocol entitled: Method Comparison and Bias Estimation Using Patient Samples; Approved Guideline (EP09-A2).

**a. Method Comparison**

A total of 138 unaltered EDTA plasma specimens were assayed in singleton utilizing the ST AIA-PACK Homocysteine assay on the AIA-2000 analyzer and the alternate method. The regression analysis for the correlation between the alternate method (x) and the ST AIA-PACK Homocysteine is as follows:

| | Deming | Regular |
|---|---|---|
| Slope: | 1.079 | 1.061 |
| Intercept: | 0.193 | 0.499 |
| Standard Error Estimate: | 2.268 | 2.257 |
| | | |
| Corr Coef( R): | 0.984 | |
| Points (Plotted/Total): | 138/138 | |
| Result Ranges: | 2.8 to 48.3 µmol/L | |

**b. Matrix comparison**

The correlation between EDTA plasma (x) and heparinized plasma (y) on the ST AIA-PACK Homocysteine was carried out using 98 unaltered specimens.

| | Deming | | Regular | |
|---|---|---|---|---|
| Slope: | 1.007 | (0.979 to 1.035) | 0.998 | (0.970 to 1.025) |
| Intercept: | -0.235 | (-0.591 to 0.121) | -0.124 | (-0.480 to 0.231) |
| Standard Error Estimate: | 0.682 | | 0.680 | |
| Corr Coef( R): | 0.991 | | | |
| Result Ranges | EDTA | 6.25 - 38-3 µmol/L | Heparin | 6.15 - 37.5 µmol/L |

The correlation between EDTA plasma (x) and serum (y) on the ST AIA-PACK Homocysteine was carried out using 98 unaltered specimens.

| | Deming | | Regular | |
|---|---|---|---|---|
| Slope: | 1.007 | (0.979 to 1.035) | 0.998 | (0.970 to 1.025) |
| Intercept: | -0.235 | (-0.591 to 0.121) | -0.124 | (-0.480 to 0.231) |
| Standard Error Estimate: | 0.682 | | 0.680 | |
| Corr Coef( R): | 0.991 | | | |
| Result Ranges | EDTA | 6.25 - 38-3 µmol/L | Heparin | 6.15 - 37.5 µmol/L |

## Specificity

The following substances were tested for cross-reactivity. The cross-reactivity (%) is the percent of the compound which will be identified as Homocysteine. If these compounds are present in the specimen at the same concentration as Homocysteine, the final result will be increased by these percentages.

| Compound | Concentration (µmol/L | Cross-reactivity (%) |
|---|---|---|
| Adenosine | 5.10 | 0.045 |
| S-Adenosyl-L-methionine | 0.474 | 1.26 |
| L-Cystathionine | 0.486 | 0.159 |
| L-Cysteine | 103 | 0.003 |
| L-Glutathione | 99.6 | 0.005 |
| L-Methionine | 0.399 | 0.059 |
| DL-Homocysteine thiolactone | 0.257 | 6.99 |

## Limit of Detection

Limit of detection: The limit of detection of the ST AIA-PACK Homocysteine was determined based on CLSI guideline EP17-A. A blank sample was measured in 60 replicates. Six low level samples were measured in 10 replicates each. As a result, the limit of detection was estimated to be 0.334 µmol/L.

The reportable range for the assay is 0.5 to 50.0 µmol/L.

## Interference

Interference is defined, for the purposes of this study, with recovery outside of 100 +/- 10% of the known concentration of the specimen after the following substances are added to human specimens. Three studies were conducted using EDTA plasma, heparinized plasma and serum.

- Hemoglobin (up to 1445 mg/dL) does not interfere with the assay
- Free bilirubin (up to 18 mg/dL) does not interfere with the assay
- Conjugated bilirubin (up to 18 mg/dL) does not interfere with the assay.
- Lipemia, as indicated by triglyceride concentration (up to 1667 mg/dL) does not interfere with the assay.
- Added protein (up to 50 mg/ml), as indicated by human g-globulin concentrations, for a total protein concentration of approximately 120 mg/ml, does not interfere with the assay.
- Ascorbic acid (up to 20 mg/dL) does not interfere with the assay
- EDTA-2K (up to 5.0 mg/mL) does not interfere with the assay.
- Heparin (up to 100 U/mL) does not interfere with the assay.

## References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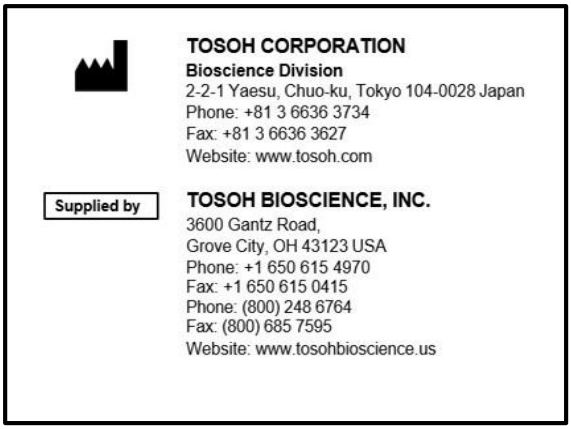
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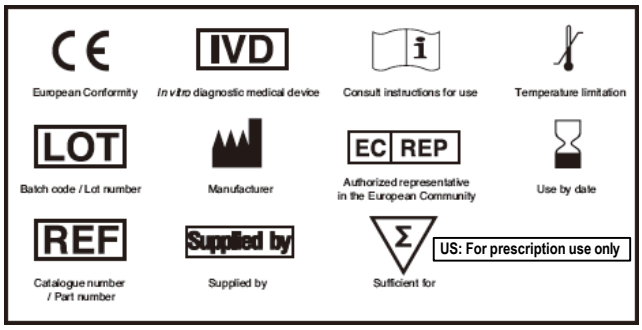

IVD
European Conformity
In vitro diagnostic medical device
Consult instructions for use
Temperature limitation
LOT
Batch code / Lot number
Manufacturer
EC REP
Authorized representative in the European Community
Use by date
REF
Catalogue number / Part number
Supplied by
Supplied by
Sufficient for
US: For prescription use only

# ST AIA-PACK HOMOCYSTEINE CALIBRATOR SET

## Intended Use

The ST AIA-PACK Homocysteine Calibrator Set is intended for IN VITRO DIAGNOSTIC USE ONLY for the calibration of the ST AIA-PACK Homocysteine assay.

## Summary and Explanation

The ST AIA-PACK Homocysteine CALIBRATOR SET contains the assigned levels of S-adenosyl-L-homocysteine. Calibration should be performed according to the schedule indicated in the Tosoh AIA System Operator's Manual.

## Material Provided (Cat. No. 0025326)

| | | | | |
|---|---|---|---|---|
| 2 x 1 mL | CALIBRATOR | (1) | 0.0 | µmol/L |

Buffered solution containing no detectable concentration of homocysteine with sodium azide as a preservative. (Ready for Use)

| | | | | |
|---|---|---|---|---|
| 2 x 1 mL | CALIBRATOR | (2) | 2 | µmol/L (approx.) |
| | | (3) | 4 | µmol/L (approx.) |
| | | (4) | 8 | µmol/L (approx.) |
| | | (5) | 15 | µmol/L (approx.) |
| | | (6) | 55 | µmol/L (approx.) |

Buffered solution containing the assigned concentration of S-adenosyl- L-homocysteine with sodium azide as a preservative. (Ready for Use)

## Warnings and Precautions

- The ST AIA-PACK Homocysteine Calibrator Set is intended for in vitro diagnostic use only.
- Inspect the packaging and the exterior of the vials for any sign of damage before use. If any damages are visible, contact your local Tosoh sales representative.
- This material contains sodium azide, which may react with lead or copper plumbing to form potentially explosive metal azides. When disposing of such reagents, always flush with large volumes of water to prevent azide build-up.
- Although material derived from human origin is not used for these calibrators, it is recommended that this product be handled with the same precautions as used for patient samples.
- Do not use beyond the expiration date.
- For safe waste disposal, it is recommended that each laboratory complies with established laboratory procedures and local, state, and federal regulations.

## Preparation and Storage

- Bring calibrator to 18 - 25 °C for use.
- Always store the ST AIA-PACK Homocysteine CALIBRATOR SET in an upright position at 2 - 8 °C when not in use.

## Stability

When stored unopened and refrigerated at 2 - 8 °C, the ST AIA-PACK Homocysteine Calibrator Set is stable until the expiration date on the label. Calibrator materials should be used within 1 day after opening provided the vials are kept tightly sealed and refrigerated at 2 - 8 °C.

## Calibration Procedure

All samples (plasma, serum, controls, and calibrators) require pretreatment prior to assaying on the Tosoh AIA System Analyzers. On the AIA-900 and AIA-2000, pretreatment is performed automatically using the parameters defined in the test file. On the AIA-360, manual pretreatment is necessary. Follow the instructions for the automatic/manual pretreatment procedure below. The pretreated samples should be assayed within 2 hours after completion of pretreatment procedure. Refer to the Tosoh AIA System Operator's Manual for additional instructions regarding pretreatment procedure.

Automatic Pretreatment Procedure

1. Bring all pretreatment reagents and samples to 18 - 25 °C
2. Using a volumetric pipette or a calibrated adjustable pipette, reconstitute the lyophilized ST AIA-PACK Homocysteine PRETREATMENT-1 accurately with 5 mL of ST AIA-PACK Homocysteine PRETREATMENT-2. Allow the lyophilized material to fully dissolve.
3. Place the reconstituted pretreatment-1 in the designated position of the reagent cassette as defined under REAGENT REGISTRATION. On the AIA-900, place the vial in the cassette and register it by the registration screen of the analyzers. On the AIA-2000, place the vial in the cassette with the barcode label facing the front.
4. Place the appropriate amount of sample cups / primary tubes, AIA-PACK SAMPLE TREATMENT CUPs and ST AIA-PACK Homocysteine test cups on the instrument.

Manual Pretreatment Procedure

1. Bring all pretreatment reagents and samples to 18 - 25 °C.
2. Using a volumetric pipette or a calibrated adjustable pipette, reconstitute the lyophilized ST AIA-PACK Homocysteine PRETREATMENT-1 accurately with 5 mL of ST AIA-PACK Homocysteine PRETREATMENT-2. Allow the lyophilized material to fully dissolve.
3. Pipette 200 µL of the reconstituted pretreatment-1 into a disposable tube.
4. Pipette 40 µL of a sample (plasma, serum, control, or calibrator) into each tube.
5. Cover the tubes and mix well using a vortex mixer.
6. Incubate the sample for 30 minutes at 18 - 25 °C.
7. Transfer all of the pretreated samples into sample cups.
8. Place the sample cups on the instrument.

9. The pretreated samples are ready for the assay with the ST AIA-PACK Homocysteine. The pretreated samples should be assayed within 2 hours after completion of pretreatment procedure. The pretreated samples should not be stored as frozen.

Calibration Procedure

Refer to the Calibration Procedure in the AIA-PACK section of this analyte application. For additional procedural instructions regarding calibration, refer to the Tosoh AIA System Operator's Manual.

1. Verify that both the calibrator lot and concentrations have been correctly entered into the analyzer software.
2. Add the appropriate amounts of each calibrator to sample cups (refer to the instrument worklist for the amount required in each sample cup.)
3. Place the sample cups and the test cups on the analyzer as indicated on the worklist.
4. Tosoh recommends that all calibrators be run in triplicate.

## Assignment of Values

The ST AIA-PACK Homocysteine Calibrator Set contains assigned concentrations of S-adenosyl -L-homocysteine. The assigned value is determined on a lot-by-lot basis and is designed to provide an assay calibration range of 0.5 to 50.0 µmol/L of homocysteine. The calibrators in this set are referred to NIST (National Institute of Standards & Technology) Standard Reference Material 1955.

## Results

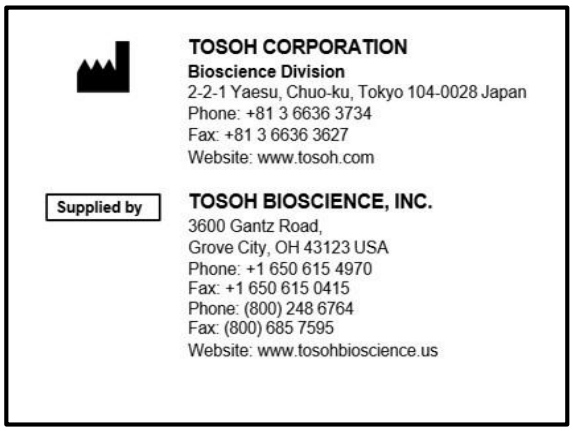
- Since there is an inverse relationship between concentration and rate, the rates should decrease as the concentration increases.
- The replicate values should be within a 10% range.

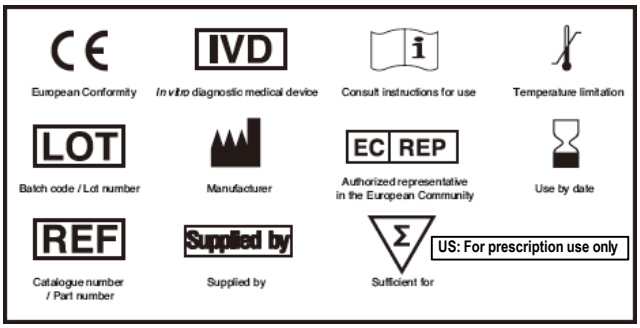
## Limitations

The ST AIA-PACK Homocysteine Calibrator Set is designed solely for use with ST AIA-PACK Homocysteine assay procedures. Although the approximate value of the highest calibrator is 55 µmol/L, the exact concentration may be slightly different. The assay specification, ASSAY RANGE HIGH, should be defined as the upper limit of the assay range, 50.0 µmol/L.

## References


1. AIA Analyte Application Manual (AAM). Tosoh Bioscience, Inc., Grove City, OH.
2. AIA-System Operator's Manual. Tosoh Bioscience, Inc., Grove City, OH.

# ST AIA-PACK HOMOCYSTEINE SAMPLE DILUTING SOLUTION

## Intended Use

The ST AIA-PACK Homocysteine SAMPLE DILUTING SOLUTION is intended for IN VITRO DIAGNOSTIC USE ONLY to dilute patient samples with concentrations of homocysteine above the upper limit of the assay range.

## Summary and Explanation

The ST AIA-PACK HOMOCYSTEINE SAMPLE DILUTING SOLUTION contains no detectable concentration of homocysteine. This Sample Diluting Solution is to be used only with samples that are being tested for homocysteine concentrations using the ST AIA-PACK HOMOCYSTEINE assay.

## Materials Provided (Cat. No. 0025526)

| | | |
|---|---|---|
| 4 x 4 mL | ST AIA-PACK Homocysteine SAMPLE DILUTING SOLUTION<br>Buffered solution containing no detectable concentration of homocysteine with sodium azide as a preservative. |

## Warnings and Precautions

- The ST AIA-PACK Homocysteine SAMPLE DILUTING SOLUTION is intended for in vitro diagnostic use only.
- Inspect the packaging and the exterior of the vials for any sign of damage before use. If any damages are visible, contact your local TOSOH sales representative.
- This material contains sodium azide, which may react with lead or copper plumbing to form potentially explosive metal azides. When disposing of such reagents, always flush with large volumes of water to prevent azide build-up.
- Although material derived from human origin is not used for the sample diluting solution, it is recommended that this product be handled with the same precautions as used for patient samples.
- Do not use beyond the expiration date.
- For safe waste disposal, it is recommended that each laboratory complies with established laboratory procedures and local, state, and federal regulations.
- After opening, the vial of the sample diluting solution should be kept tightly sealed with a clean rubber cap. Sealing with dirty material may cause deterioration of the reagent.
- The remaining sample diluting solution after use should not be mixed with another vial

## Preparation and Storage

- The ST AIA-PACK Homocysteine SAMPLE DILUTING SOLUTION is provided ready for use.
- Always store the Sample Diluting Solution in an upright position at 2 - 8 °C when not in use.
- The sample diluting solution should be used after equilibrating to 18 - 25 °C for about 30 minutes.

## Stability

When stored unopened and refrigerated at 2 - 8 °C, the ST AIA-PACK HOMOCYSTEIME SAMPLE DILUTING SOLUTION is stable until the expiration date on the label.

The in-use stability of the ST AIA-PACK Homocysteine Sample Diluting Solution is 9 days provided: 1) it is used for automatic dilutions, 2) it is at 18 - 25 °C for only 8 hours per day, and 3) the vials are closed and kept refrigerated immediately after use. In-use stability is 90 days provided: 1) it is used for manual dilutions ONLY, and 2) the vials are closed and refrigerated immediately after use.

## Procedure

Refer to the AIA System Operator's Manual for additional procedural instructions regarding sample dilution.

1. If a specimen homocysteine concentration is found to be greater than the upper limit of the assay range, 50.0 µmol/L, the specimen should be diluted with the ST AIA-PACK Homocysteine SAMPLE DILUTING SOLUTION and assayed according to the PROCEDURE in the insert sheet of the ST AIA-PACK Homocysteine.
2. The AIA-900 and AIA-2000 will perform dilutions automatically if the dilution factors are entered into the software prior to assaying the diluted sample.
3. The recommended dilution for specimens containing greater than 50.0 µmol/L is 5 times. It is desirable to dilute the specimen so that the diluted specimen reads between 0.5 and 50.0 µmol/L.

## Results

When an auto-dilution is performed, the AIA instrument will calculate the final result.

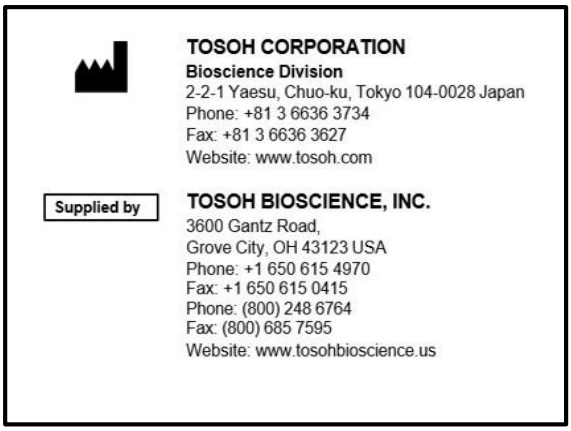
## Limitations

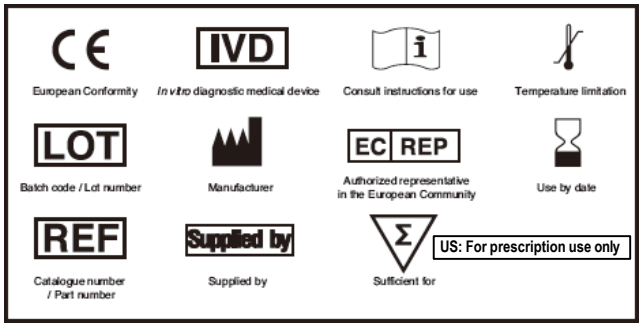
The ST AIA-PACK HOMOCYSTEINE SAMPLE DILUTING SOLUTION is designed solely for use with ST AIA-PACK assay procedures.

The assay specification, ASSAY RANGE HIGH, should be defined as the upper limit of the assay range, 50.0 µmol/L.

## References


1. AIA Analyte Application Manual (AAM). Tosoh Bioscience, Inc., Grove City, OH.
2. AIA-System Operator's Manual. Tosoh Bioscience, Inc., Grove City, OH.

# ST AIA-PACK HOMOCYSTEINE PRETREATMENT SET

## Intended Use

The ST AIA-PACK Homocysteine PRETREATMENT SET is intended for IN VITRO DIAGNOSTIC USE ONLY for the pretreatment of samples (plasma, serum, controls, and calibrators) for the ST AIA-PACK Homocysteine assay.

## Summary and Explanation

The ST AIA-PACK Homocysteine PRETREATMENT SET is designed to convert the Homoscysteine in samples (hemolysate mixtures, calibrators, controls) to the measurable form for this analyte. The pretreatment should be performed according to the protocol described in the PROCEDURE section in this insert.

## Materials Provided (Cat. No. 0025726)

PRETREATMENT-1

6 x 5.0 mL ST AIA-PACK Homocysteine PRETREATMENT-1

Lyophilized material containing S-adenosyl-L-homocysteine hydrolase.

PRETREATMENT-2

6 x 5.0 mL ST AIA-PACK Homocysteine PRETREATMENT-2

Buffered solution containing tris (2-carboxyethyl) phosphine (TCEP), adenosine with sodium azide as a preservative. (Ready to Use)

## Warnings and Precautions

- The ST AIA-PACK Homocysteine PRETREATMENT SET is intended for in vitro diagnostic use only.
- Inspect the packaging and the exterior of the vials for any sign of damage before use. If any damages are visible, contact your local Tosoh sales representative.
- The ST AIA-PACK Homocysteine PRETREATMENT-2 contains sodium azide, which may react with lead or copper plumbing to form potentially explosive metal azides. When disposing of such reagents, always flush with large volumes of water to prevent azide build-up.
- Although material derived from human origin is not used for these pretreatment reagents, it is recommended that this product be handled with the same precautions as used for patient samples.
- Pretreated patient samples should be handled with the same precautions as used for the samples before pretreatment.
- The pretreatment sets with different lot numbers shall not be mixed.
- Do not pool the remaining pretreatment reagents after use.
- Do not use beyond the expiration date.

- For safe waste disposal, it is recommended that each laboratory complies with established laboratory procedures and local, state, and federal regulations.

## Preparation and Storage

- Bring all pretreatment reagents to 18 - 25 °C.
- Using a volumetric pipette or a calibrated adjustable pipette, reconstitute the lyophilized ST AIA-PACK Homocysteine PRETREATMENT-1 accurately with 5 mL of ST AIA-PACK Homocysteine PRETREATMENT-2.
- Allow the lyophilized material to fully dissolve.
- Always store the Pretreatment Set in an upright position at 2 - 8 °C when not in use.

## Stability

When stored unopened and refrigerated at 2 - 8 °C, the ST AIA-PACK Homocysteine PRETREATMENT SET is stable until the expiration date on the label. The in-use stability of the ST AIA-PACK Homocysteine Pretreatment Reagent is stable at 18 - 25 °C for 20 hours. The in-use stability is stable at 2 - 8 °C for 1 day provided: 1) it is used for manual pretreatment ONLY, and 2) the bottles are closed and refrigerated immediately after use.

## Procedure

**NOTE:** All samples (plasma, serum, controls, and calibrators) require pretreatment prior to assaying on the Tosoh AIA System Analyzers. On the AIA-900 and AIA-2000, pretreatment is performed automatically using the parameters defined in the test file. On the AIA-360, manual pretreatment is necessary. Follow the instructions for the automatic/manual pretreatment procedure below. The pretreated samples should be assayed within 2 hours after completion of pretreatment procedure. Refer to the Tosoh AIA System Operator's Manual for additional instructions regarding pretreatment procedure.

### Automatic Pretreatment Procedure

1. Bring all Pretreatment Reagents and samples to 18 - 25 °C.
2. Using a volumetric pipette or a calibrated adjustable pipette, reconstitute the lyophilized ST AIA-PACK Homocysteine PRETREATMENT-1 accurately with 5.0 mL of ST AIA-PACK Homocysteine PRETREATMENT-2. Allow the lyophilized material to fully dissolve.
3. Place the reconstituted pretreatment-1 in the designated position of the reagent cassette as defined under REAGENT REGISTRATION. On the AIA-900, place the vial in the cassette and register it by the registration screen of the analyzers. On the AIA-2000, place the vial in the cassette with the barcode label facing the front.
4. Place the appropriate amount of sample cups / primary tubes, AIA-PACK SAMPLE TREATMENT CUPs and ST AIA-PACK Homocysteine test cups on the instrument.

### Manual Pretreatment Procedure

1. Bring all pretreatment reagents and samples to 18 - 25 °C.
2. Using a volumetric pipette or a calibrated adjustable pipette, reconstitute the lyophilized ST AIA-PACK Homocysteine PRETREATMENT-1 accurately with 5 mL of ST AIA-PACK Homocysteine PRETREATMENT-2. Allow the lyophilized material to fully dissolve.

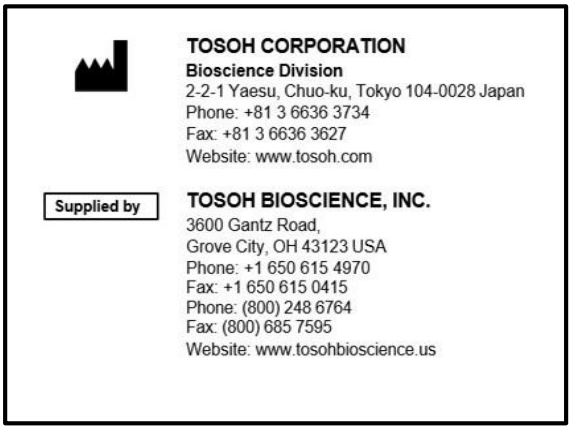
3. Pipette 200 µL of the reconstituted pretreatment-1 into a disposable tube.
4. Pipette 40 µL of a sample (plasma, serum, control, or calibrator) into each tube.
5. Cover the tubes and mix well using a vortex mixer.
6. Incubate the sample for 30 minutes at 18 - 25 °C.
7. Transfer all of the pretreated samples into sample cups.
8. Place the sample cups on the instrument.
9. The pretreated samples are ready for the assay with the ST AIA-PACK Homocysteine. The pretreated samples should be assayed within 2 hours after completion of pretreatment procedure. The pretreated samples should not be stored as frozen.

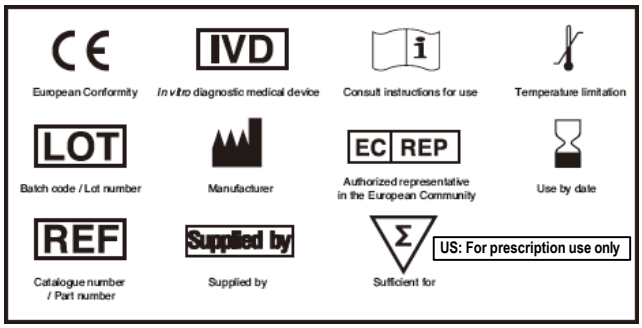
## Limitations

The ST AIA-PACK Homocysteine PRETREATMENT SET is designed solely for use with ST AIA-PACK Homocysteine assay procedures.

## References


1. AIA Analyte Application Manual (AAM). Tosoh Bioscience, Inc., Grove City, OH.
2. AIA System Operator's Manual. Tosoh Bioscience, Inc., Grove City, OH.

# AIA-PACK HOMOCYSTEINE CONTROL SET

## Intended Use

The AIA-PACK Homocysteine Control Set is intended for IN VITRO DIAGNOSTIC USE ONLY for performing quality control procedures with the ST AIA-PACK Homocysteine assay.

**CL AIA-PACK values listed on the Control product labeling (Box and Vials) are for International Use Only and are not intended for use with products in the United States.**

## Summary and Explanation

The AIA-PACK Homocysteine Control Set contains buffered bovine serum albumin with the assigned levels of homocysteine. The homocysteine controls shall be run at the beginning of each day on which homocysteine assays are scheduled.

## Materials Provided (Cat. No. 0025426)

| | |
|---|---|
| 2 x 4 mL | AIA-PACK Homocysteine Control LEVEL 1 - Buffered bovine serum albumin containing approximately 12 µmol/L of homocysteine with sodium azide as a preservative. See the vial label for the assigned concentration range. (Ready to Use) |
| 2 x 4 mL | AIA-PACK Homocysteine Control LEVEL 2 - Buffered bovine serum albumin containing approximately 25 µmol/L of homocysteine with sodium azide as a preservative. See the vial label for the assigned concentration range. (Ready to Use) |

## Warnings and Precautions

- The AIA-PACK Homocysteine Control Set is intended for in vitro diagnostic use only.
- Inspect the packaging and the exterior of the vials for any sign of damage before use. If any damages are visible, contact your local Tosoh sales representative.
- This material contains sodium azide, which may react with lead or copper plumbing to form potentially explosive metal azides. When disposing of such reagents, always flush with large volumes of water to prevent azide build-up.
- Although material derived from human origin is not used for these controls, it is recommended that this product be handled with the same precautions as used for patient samples.
- Do not use beyond the expiration date.
- For safe waste disposal, it is recommended that each laboratory complies with established laboratory procedures and local, state, and federal regulations.

## Preparation and Storage

The AIA-PACK Homocysteine Control Set is provided ready for use. Bring the controls to 18 - 25 °C for use. Always store the AIA-PACK Homocysteine CONTROL SET in an upright position at 2 - 8 °C when not in use.

## Stability

When stored unopened and refrigerated at 2 - 8 °C, the control set is stable until the expiration date on the label. The in-use stability of the AIA-PACK Homocysteine Control Set is 14 days at 2 - 8 °C. If stored at 18 - 25 °C the in-use stability is 1 day.

## Procedure

All samples (plasma, serum, controls, and calibrators) require pretreatment prior to assaying on the Tosoh AIA System Analyzers. On the AIA-900 and AIA-2000, pretreatment is performed automatically using the parameters defined in the test file. On the AIA-360, manual pretreatment is necessary. Follow the instructions for the automatic/manual pretreatment procedure below. The pretreated samples should be assayed within 2 hours after completion of pretreatment procedure. Refer to the Tosoh AIA System Operator's Manual for additional instructions regarding pretreatment procedure.

### Automatic Pretreatment Procedure

1. Bring all pretreatment reagents and samples to 18 - 25 °C.
2. Using a volumetric pipette or a calibrated adjustable pipette, reconstitute the lyophilized ST AIA-PACK Homocysteine PRETREATMENT-1 accurately with 5 mL of ST AIA-PACK Homocysteine PRETREATMENT-2. Allow the lyophilized material to fully dissolve.
3. Place the reconstituted pretreatment-1 in the designated position of the reagent cassette as defined under REAGENT REGISTRATION. On the AIA-900, place the vial in the cassette and register it by the registration screen of the analyzers. On the AIA-2000, place the vial in the cassette with the barcode label facing the front.
4. Place the appropriate amount of sample cups / primary tubes, AIA-PACK SAMPLE TREATMENT CUPs and ST AIA-PACK Homocysteine test cups on the instrument.

### Manual Pretreatment Procedure

1. Bring all pretreatment reagents and samples to 18 - 25 °C.
2. Using a volumetric pipette or a calibrated adjustable pipette, reconstitute the lyophilized ST AIA-PACK Homocysteine PRETREATMENT-1 accurately with 5 mL of ST AIA-PACK Homocysteine PRETREATMENT-2. Allow the lyophilized material to fully dissolve.
3. Pipette 200 µL of the reconstituted pretreatment-1 into a disposable tube.
4. Pipette 40 µL of a sample (plasma, serum, control, or calibrator) into each tube.
5. Cover the tubes and mix well using a vortex mixer.
6. Incubate the sample for 30 minutes at 18 - 25 °C.
7. Transfer all of the pretreated samples into sample cups.
8. Place the sample cups on the instrument.
9. The pretreated samples are ready for the assay with the ST AIA-PACK Homocysteine. The pretreated samples should be assayed within 2 hours after completion of pretreatment procedure. The pretreated samples should not be stored as frozen.

## Quality Control Procedure

Refer to the Tosoh AIA System Operator's Manual for additional procedural instructions regarding quality control.

1. Load the appropriate amount of test cups on the instrument.
2. Add the appropriate amounts of each control to sample cups (refer to the instrument worksheet for the sample volume).
3. Print a work list and place the sample cups in the position indicated.

# Limitations

The AIA-PACK Homocysteine Control Set is designed solely for use with ST AIA-PACK Homocysteine assay procedures.

# References


1. AIA Analyte Application Manual (AAM). Tosoh Bioscience, Inc., Grove City, OH.
2. AIA System Operator's Manual. Tosoh Bioscience, Inc., Grove City, OH.