Human Homocysteine ELISA Kit

Cat. No.: DEIA1724
Pkg. Size: 96T

Intended use
The Homocysteine ELISA is intended for measurement of total L-Homocysteine in human serum or plasma.

General Description
Homocysteine is a non-protein amino acid with the formula HSCH2CH2CH(NH2)CO2H. It is a homologue of the amino acid cysteine, differing by an additional methylene (-CH2-) group. It is biosynthesized from methionine by the removal of its terminal Cε methyl group. Homocysteine can be recycled into methionine or converted into cysteine with the aid of B-vitamins.

Principle Of The Test
The following solid-phase enzyme immunoassay is based on competition between SAH in the sample and immobilised SAH bound to the walls of the microtitre plate for binding sites on a monoclonal anti-SAH antibody. After removal of unbound anti-SAH antibody, a secondary rabbit anti-mouse antibody labelled with the enzyme horse radish peroxidase (HRP) is added. The peroxidase activity is measured spectrophotometrically after addition of substrate, and the absorbance is inversely related to the concentration of Hcy in the sample.

Reagents And Materials Provided

- [REAG A] Assay Buffer: 54 mL
- [REAG B] Adenosine/DTT: 3.5 mL
- [REAG C] SAH-hydrolase: 3.5 mL
- [REAG D] Enzyme Inhibitor: 55 mL
- [REAG E] Adenosine deaminase: 55 mL
- [REAG F] a-SAH Antibody: 25 mL
- [REAG G] Enzyme Conjugate: 15 mL
- [REAG H] Substrate Solution: 15 mL
- [REAG S] Stop Solution: 20 mL
- [BUF WASH] Wash Buffer: 60 mL
- [CAL 1] – [CAL6] Standards: 6 x 1.5 mL
- [MICROTITER STRIPS] Microtiter wells: 12 x 8 wells
- [CONTROL L]: 1.5 mL
- [CONTROL M]: 1.5 mL
- [CONTROL H]: 1.5 mL
- [BUF WASH]: 1000 mL

Materials Required But Not Supplied

1. Homocysteine controls (see section “Quality Controls” for more information)
2. Plastic or glass tubes for pre-treatment of samples
3. Pipettes / multipipettes 25 μL, 100 μL, 200 μL and 500 μL or 8 channel multipipette for 100 μL and 200 μL
4. Volumetric flask 50 mL and 600 mL
5. Incubator, 37 °C
6. Washer and reader (450 nm) for microtiter plates

Specimen Collection And Handling

EDTA-plasma or serum may be used with the Homocysteine ELISA assay. As synthesis of Hcy continues in red blood cells after drawing, it is very important to prepare specimens as follows:

1. Serum samples should be allowed to clot for no more than 30 minutes before centrifugation and separation of serum. Serum samples should be kept on ice prior to separation.
2. EDTA-plasma samples must be centrifuged or put on ice immediately after drawing. EDTA-plasma samples may be kept on ice for up to 6 hours prior to separation by centrifugation.

Food consumption can affect circulating Homocysteine levels. Protein rich meals give higher Homocysteine values and should be avoided late in the day before sampling.

Standardised sampling procedures are crucial due to the above mentioned influencing factors. Complete mixing of thawed samples is required before use.

Plasma or serum samples may be stored for 12 weeks at 2 - 8 °C, for up to 3 weeks at room temperature (18 - 25 °C) and have been shown to be stable for at least 8 months if frozen at minus 20 °C.

Reagent Preparation

1. Components should be stored refrigerated (2 - 8 °C). Store all bottles upright and tightly capped. The components are stable until the stated expiration date when stored and handled as directed. Once the components in the Homocysteine ELISA Kit are opened they are stable for 12 weeks when stored at 2-8 °C.
2. The sample pre-treatment solution has to be made by mixing Reagent A, B and C. The solution is stable for one hour and has to be freshly made for each run.
3. The Wash Buffer must be diluted (1±9) with distilled water before use. The prepared Wash buffer is stable for 4 weeks when stored at room temperature (18-25 °C).
4. Reagent D and H are stored in dark bottles to avoid exposure to light.
5. It is important that the microtiter strips are kept dry, i.e. in the sealed bag with drying capsules, and stored refrigerated. Equilibration for a minimum of two hours is required to reach room temperature (18-25 °C). Leave the strips in the bag during equilibration.
6. Only the necessary number of microtiter strips should be kept in the frame during the run. Unused strips should be kept in the sealed bag with drying capsules.
7. Avoid exposure of the kit to temperatures exceeding 37 °C as this may denature the enzymes.

Assay Steps

Make sure all solutions and microtiter strips are equilibrated to room temperature before use. Leaving the kit at room temperature over night is recommended. We recommend running the standards in duplicate and to performing a new calibration curve for each run to avoid run-to-run variations using coated microtiter plates.

Sample pre-treatment procedure

1. Sample pre-treatment solution must be made up no more than 1 hour prior to the start of the assay. Volume needed per 10 samples (no dead volume calculated):
   - 4.5 mL REAG A
   - 0.25 mL REAG B
   - 0.25 mL REAG C Mix.
2. Dilute calibrators and samples/controls in plastic or glass tubes as follows:
   - 25 μL standard/sample/control ± 500 μL sample pre-treatment solution Mix well.
   - Incubate for 30 minutes at 37 °C (Cap the tubes or cover with parafilm during incubation).
   - Note: Proceed with step 3 before the samples have cooled.
3. Add 500 μl REAG D  
Mix well. Incubate for 15 minutes at 18-25 °C.

4. Add 500 μl REAG E  
Mix well. Incubate for 5 minutes at 18-25 °C.

**Microtitre Plate procedure**

5. Pipette 25 μl diluted calibrator / sample / control from step 4 into the wells of the SAH-coated microtitre strips.

6. Add 200 μl REAG F to each well. Incubate for 30 min at 18-25 °C.

Use the enclosed lid during all incubations.

7. Wash with diluted Wash buffer.

Use 3 x 400 μl. If manual washing is required, use 4 times 350 μl instead of 3 times 400 μl. After washing, empty the wells on paper towels.

8. Add 100 μl REAG G to each well. Incubate for 20 min at 18-25 °C.

9. Wash with diluted Wash buffer.

Use 3 x 400 μl. If manual washing is required, use 4 times 350 μl instead of 3 times 400 μl. After washing, empty the wells on paper towels.

10. Add 100 μl REAG H to each well. Incubate for 10 min at 18-25 °C.

11. Add 100 μl REAG S to each well.

12. Shake and read at 450 nm within 15 minutes (Automatic plate shaker is preferred to ensure proper mixing).

**Quality Control**

We recommend that each laboratory use a Homocysteine control with known value.

**Precautions**

1. Reagent D contains 0.15% merthiolate (≤ 0.074% mercury), and is classified as “Harmful”. Please handle and dispose of properly (See section “Product Safety Information”).

2. 0.01% merthiolate is used as preservative in some reagents. Each kit contains less than 0.028% mercury. Please handle and dispose of appropriately.


4. Reagent S contains 0.8M sulphuric acid, and is classified as “Irritant”. Please handle and dispose of properly (See section “Product Safety Information”).

5. Calibrators, Controls, Reagent A and Reagent E contain less than 0.10% sodium azide as preservative. Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. On disposal, flush with a large volume of water to prevent azide build-up.

6. Controls contain sera originating from human blood samples. The source materials have been tested and found to be negative for Hepatitis B Surface Antigen (HBsAg), HIV-1 Antigen (HIVAg), HCV antibody, HIV-1/2 antibody, HTLV-1/2 antibody and Hepatitis B core Antibody (Hbc). However, blood derivatives should be handled according to recommended procedures for handling infectious material. HHS publication no. (CDC) 93-8395 [18] or local/national guidelines on laboratory safety procedures should be consulted.

7. Reagents with different lot numbers must not be interchanged.

8. Do not use the kit after the expiration date on the outer box.

**Limitations**

1. If an automatic pipetting station is used, thorough washing of the tubing after addition of the blue coloured Reagent G may be needed - preferably with diluted acid followed by water. Any remaining solution in the tubing will interfere with the next assay step; i.e. addition of Reagent H.

2. The washing procedure is critical for obtaining good precision. If manual washing is required, use 4 times 350 μl instead of 3
times 400 μL. After washing, empty the wells on paper towels.

3. Avoid exposure of the kit to temperatures exceeding 37 °C as this may denature the enzymes.

4. Specimens from donors who are on drug therapy involving S-adenosyl-methionine may show falsely elevated levels of Homocysteine.

5. Specimens samples from donors who have received preparations of mouse monoclonal antibodies for diagnosis or therapy may contain human anti-mouse antibody (HAMA). HAMA, present in serum or plasma specimens, may interfere in immunoassays which utilise mouse monoclonal antibodies. These specimens should not be assayed with the Homocysteine ELISA assay.

6. Specimens from donors taking methotrexate, carbamazepine, phenytoin, nitrous oxide, anti-convulsants or 6-azauridine triacetate, may have elevated levels of Homocysteine due to metabolic interference with the Homocysteine metabolism.

REFERENCES


