DRG® Homocysteine (EIA-2925)

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Revised 3 Mar. 2011 rm (Vers. 3.1)

Please use only the valid version of the package insert provided with the kit.

This kit is intended for Research Use Only.

Not for use in diagnostic procedures.

Intended Use

The Homocysteine ELISA is intended for determination of total L-homocysteine in human serum or plasma.

Assay Principle

The Homocysteine ELISA is an enzyme immunoassay for the determination of Hcy in blood.[16] Protein-bound Hcy is reduced to free Hcy and enzymatically converted to S-adenosyl-L-homocysteine (SAH) in a separate procedure prior to the immunoassay.[17] The enzyme is specific for the L-form of homocysteine, which is the only form present in the blood.

Reduction

Hcy, mixed disulfide and protein-bound forms of Hcy in the sample are reduced to free Hcy by use of dithiothreitol (DTT).

Prot-SS-Hcy
*R1-SS- Hcy —DTT→ Hcy (*R1 is any thiol-residue.)
Hcy-SS-Hcy

Enzymatic conversion

Hcy in the test sample is converted to S-adenosyl-L-homocysteine by the use of SAH hydrolase and excess adenosine (Ad).

Hcy + Ad —SAH-hydrolase→ SAH + H_2O

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 absor¬bance is inversely related to the concentration of Hcy in the sample.

Warnings and 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”).

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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.
3. Reagent F contains mouse antibodies and Reagent G contains rabbit antibodies.
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.

Kit Components
Homocysteine ELISA Kit, 96 wells

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Kit Components	Solution	Cap Code	Component Description	Volume
[REAG A]	Assay Buffer	A	Phosphate buffer, sodium azide	54 mL
[REAG B]	Adenosine/DTT	B	Adenosine / dithiothreitol, citric acid	3.5 mL
[REAG C]	SAH-hydrolase	C	Recombinant S-adenosyl-L-homocysteine hydrolase, trisbuffer, glycerol, methylparaben	3.5 mL
[REAG D]	Enzyme Inhibitor	D	Merthiolate, phosphate buffer	55 mL
[REAG E]	Adenosine deaminase	E	Adenosine deaminase, phosphate buffer, sodium azide, BSA, phenol-red dye	55 mL
[REAG F]	a-SAH Antibody	F	Monoclonal mouse-anti-S-adenosyl-L-homocysteine antibody, BSA, merthiolate	25 mL
[REAG G]	Enzyme Conjugate	G	Rabbit anti-mouse-antibody enzyme conjugate, BSA, horse radish peroxidase, blue dye	15 mL
[REAG H]	Substrate Solution	H	N-methyl-2-pyrrolidon, propyleneglycol	15 mL
[REAG S]	Stop Solution	S	0.8 M Sulphuric acid	20 mL
[BUF WASH]	Wash Buffer	W	Phosphate buffer, merthiolate, Tween 20, BSA	60 mL
[CAL 1] – [CAL 6]	Standards	1-6	S-adenosyl-L-homocysteine (2, 4, 8, 15, 30, 50 µmol/L) in buffer with preservative	6 x 1.5 mL
[MICROTITER STRIPS]	Microtiter wells	-	Coated with S-adenosyl-L-homocysteine	12 x 8 wells

Wash Buffer is concentrated and must be diluted (1+9) with purified water before use.
All other components are ready to use.

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Homocysteine ELISA Control kit (EIA-3329)

Kit Components	Cap Code	Component Description	Volume
[CONTROL L]	L	7.0 µmol/L homocysteine in diluted serum samples of human origin, phosphate buffer and preservative	1.5 mL
[CONTROL M]	M	12.5 µmol/L homocysteine in diluted serum samples of human origin, phosphate buffer and preservative	1.5 mL
[CONTROL H]	H	25.0 µmol/L homocysteine in diluted serum samples of human origin, phosphate buffer and preservative	1.5 mL

All controls are ready to use.

Homocysteine ELISA Wash Buffer

Kit Components	Cap Code	Component Description	Volume
[BUF WASH]	W	Phosphate buffer, merthiolate, Tween 20, BSA	1000 mL

[BUF WASH] is concentrated and must be diluted (1+9) with purified water before use.

Materials required but not provided in the kit:

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

Preparation and Storage of Kit Components

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 (see Section "Procedure"). 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.

Specimen Collection and Preparation

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:

- 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.
- 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.[19, 20]
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.

Limitations

- 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.
- 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.
- Avoid exposure of the kit to temperatures exceeding 37 °C as this may denature the enzymes.
- Specimens from donors who are on drug therapy involving S-adenosyl-methionine may show falsely elevated levels of homocysteine.

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- Specimens from donors who have received preparations of mouse monoclonal antibodies 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.
- 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.

Procedure

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.

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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).

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