

Competitive ELISA Kit

User Instruction

Human Formylmethionine ELISA Kit

Cat.NO AMS.EA1893Hu

This product is for research use only, not for use in diagnosis procedures. It's highly recommend to read this instruction entirely before the use. More details please visit: www.bt-laboratory.com

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Precision

Intra-Assay Precision (Precision within an assay) Three samples of known concentration were tested on one plate to assess intra-assay precision.

Inter-Assay Precision (Precision between assays) Three samples of known concentration were tested in separate assays to assess inter-assay precision.

$$CV(\%) = SD/\text{mean} \times 100$$

Intra-Assay: CV < 10%

Inter-Assay: CV < 12%

Standard Curve Range: 2nmol/ml - 600nmol/ml

Sensitivity: 1.11nmol/ml

Size: 96 wells

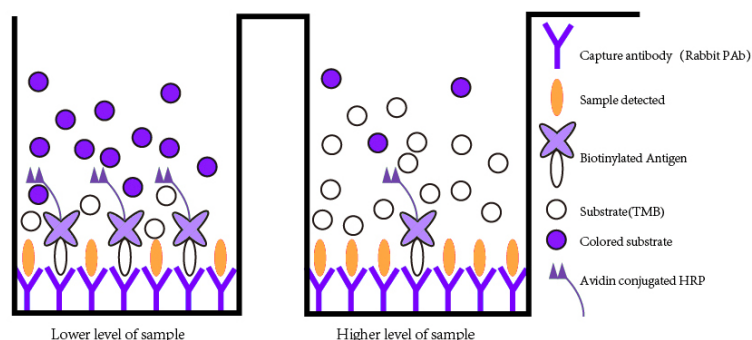
Storage: Store the reagents at 2-8°C. For long term storage refer to the expiration date keep it at -20°C. Avoid repeated thaw cycles. If individual reagents are opened it is recommended that the kit be used within 1 month.

Intended Use

This competitive kit is for the quantification of Formylmethionine(also known as fMet) in serum, plasma, cell culture supernates, cell lysates, tissue homogenates.

Assay Principle

This kit is a Enzyme-Linked Immunosorbent Assay (ELISA). fMet standards or samples are added to the wells pre-coated with a monoclonal antibody. Then biotin-conjugated target antigen are added to the wells. The antigens in the standards or samples compete with the biotin-conjugated antigen to bind to the capture antibody and incubate. Unbound antigen is washed away during a washing step. An avidin-HRP is then added and then incubate. Unbound avidin-HRP is washed away during a washing step. TMB Substrate is then added and color develops. The reaction is stopped by addition of acidic stop solution and color changes into yellow that can be measured at 450 nm. The intensity of the color developed is inversely proportional to the concentration of fMet in the sample. The concentration of fMet in the sample is then determined by comparing the O.D. of the samples to the standard curve.



Reagents Provided

Components	Quantity
Pre-coated Plate	12 * 8 well strips x 1
Human fMet Standard, lyophilized	150µl x 2 vials
Standard/Sample Diluent	6ml x 1 vial
Biotinylated Antigen, lyophilized	1 vial
Avidin-HRP Concentrate	100µl x 1 vial
Biotinylated Antigen Diluent	6ml x 1 vials
Avidin HRP Diluent	5.9ml x 1 vials
Substrate Solution A	6ml x 1 vial
Substrate Solution B	6ml x 1 vial
Stop Solution	6ml x 1 vial
Wash Buffer Concentrate (25x)	20ml x 1 vial
Plate Sealer	2 pcs
Zipper Bag	1
User Instruction	1

Materials Required But Not Supplied

- 37°C±0.5°C incubator
- Precision pipette and disposable tip
- Deionized or distilled water
- Clean tube
- Absorbent paper
- Microplate reader with 450 ± 10nm wavelength filter

Precautions

- Prior to running the assay, the kit and sample should be warmed naturally to room temperature 30 minutes.
- Once the desired number of strips has been removed, immediately reseal the bag to protect the remain from deterioration. Cover all reagents when not in use.
- Make sure pipetting order and rate of addition from well-to-well when pipetting reagents.
- There are two vials of standard in the kit for users, please cover the other unused vial and keep refrigerated.
- Do not allow wells to become dry during the assay procedure.
- This instruction should be strictly followed in the experiment.
- Pipette tips and plate sealer in hand should be clean and disposable to avoid cross-contamination.
- Avoid using the reagents from different batches together.
- Substrate solution B is sensitive to light, don't expose substrate solution B to light for a long time.
- Stop solution contains acid. Please wear eye, hand and skin protection when using this material. Avoid contact of skin or mucous membranes with kit reagent.
- The kit should not be used beyond the expiration date.

Specimen Collection

Serum Allow serum to clot for 10-20 minutes at room temperature. Centrifuge at 2000-3000 RPM for 20 minutes.

Plasma Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge samples for 15 minutes at 2000-3000 RPM at 2-8°C within 30 minutes of collection.

Urine Collect by sterile tube. Centrifuge at 2000-3000 RPM for approximately 20 minutes. When collecting pleuroperitoneal fluid and cerebrospinal fluid, please follow the procedures above-mentioned.

Cell culture supernatant Collect by sterile tubes when examining secrete components. Centrifuge at 2000-3000 RPM for approximately 20 minutes. Collect the supernatants carefully. When examining the components within the cell, use PBS (pH 7.2-7.4) to dilute cell suspension to the cell concentration of approximately 1 million/ml. Damage cells through repeated freeze-thaw cycles to let out the inside components. Centrifuge at 2000-3000 RPM for approximately 20 minutes.

Tissue Rinse tissues in PBS (pH 7.4) to remove excess blood thoroughly and weigh before homogenization. Mince tissues and homogenize them in PBS (pH7.4) with a glass homogenizer on

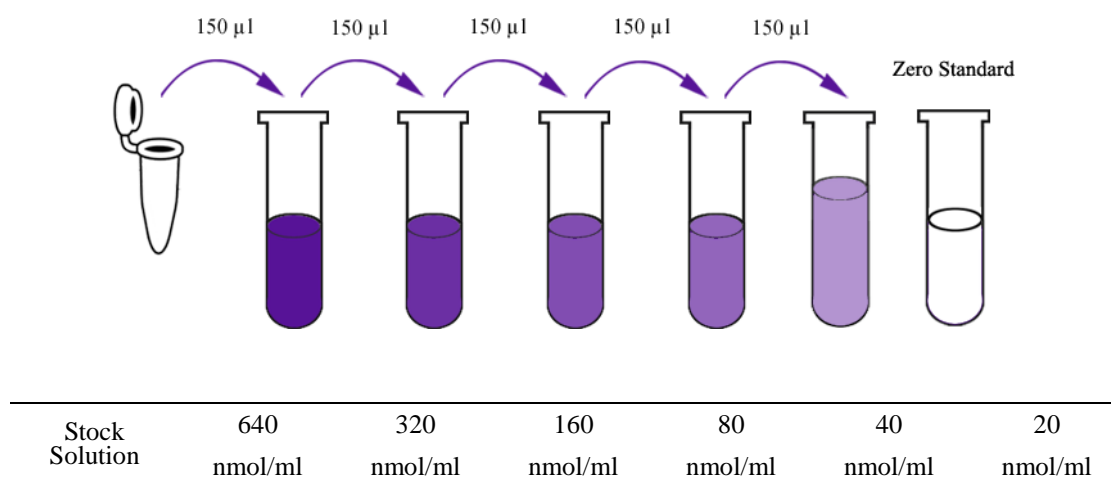
ice. Thaw at 2-8°C or freeze at -20°C. Centrifuge at 2000-3000 RPM for approximately 20 minutes.

Note

- The supplier is only responsible for the kit itself, but not for the samples consumed during the assay. The user should calculate the possible amount of the sample used in the whole test.
- Samples to be used within 5 days should be stored at 2-8°C. Samples should be aliquoted or must be stored at -20°C within 1 month or -80°C within 6 months. Avoid repeated freeze thaw cycles.
- Samples should be brought to room temperature before starting the assay.
- Samples containing NaN₃ can not be tested as it inhibits the activity of HRP.
- Collect the supernatants carefully. When sediments occurred during storage, centrifugation should be performed again.
- Hemolysis can greatly impact the validity of test results. Take care to minimize hemolysis.

Reagents Preparation

- All reagents should be brought to room temperature before use.
- **Standard** Reconstitute the standard with 150 µl of standard diluent to generate a 640nmol/ml standard stock solution which should be used within 24 hours. Allow the standard to sit for 15 minutes with gentle agitation prior to making dilutions. Prepare duplicate or triplicate standard points by serially diluting the standard stock solution 1: 2 with diluent to produce 320nmol/ml, 160nmol/ml, 80nmol/ml, 40nmol/ml and 20nmol/ml solutions. The sample diluent serves as the zero standard (0 nmol/ml). If the solutions haven't been run out, the remain should be stored at -20°C and used within one month.



- **Biotinylated Antigen** Briefly centrifuge the biotinylated antigen vial and reconstitute with diluent to produce a stock solution. And then pipette this stock solution into the diluent vial.

Mix well. Allow to sit for 10 minutes with gentle agitation prior to making dilutions. If the solutions haven't been run out, the remain should be stored at -20°C and used within one month.

- **Avidin-HRP Concentrate** Briefly low- speed centrifuge the avidin-HRP and then pipette avidin-HRP into the diluent vial. Mix well. Allow to sit for 10 minutes with gentle agitation prior to making dilutions.
- **Wash Buffer Concentrate 25x** Dilute the concentrated wash buffer with distilled water to yield 500 ml of 1x wash buffer. If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved. Dilute the Wash Buffer Concentrate 1: 25 with reagent grade water.

Assay Procedure

1. Prepare all reagents, standard solutions and samples as instructed. Bring all reagents to room temperature before use. The assay is performed at room temperature.
2. Determine the number of strips required for the assay. Insert the strips in the frames for use. The unused strips should be stored at 2~8°C for up to one month.
3. Add 50 µl standard or sample well, and add 50 µl biotinylated antigen to each well (Not blank control well) Mix well. Cover the plate with a sealer and incubate for 30 minutes.
4. Wash five times with 300 µl wash buffer manually. Invert the plate each time and decant the contents; hit 4-5 times on absorbent material to complete remove liquid. For automated washing, aspirate all wells and wash 5 times with wash buffer, overfilling wells with wash buffer. Blot the plate on absorbent material.
5. Add 50 µl concentrated avidin-HRP to standard well and sample well and incubate for 30 minutes and cover the plate with a sealer.
6. Remove the sealer and wash as described above.
7. Add 50 µl substrate solution A to each well and then add 50 µl substrate solution B to each well. Incubate plate covered with a new sealer for 10 minutes at 37°C in the dark.
8. Add 50 µl Stop Solution to each well, the blue color will change into yellow immediately.
9. Determine the optical density (OD value) of each well immediately using a microplate reader set to 450 nm within 15 minutes after adding the stop solution.

Summary

Prepare all reagents, samples and standards.

Add samples, standards and biotinylated antigen.
Incubate for 30 minutes at 37°C.

Aspirate and wash 5 times.

Add avidin-HRP and incubate for 30 minutes at 37°C.

Aspirate and wash 5 times.

Add substrate solution A and substrate solution B and
incubate in the dark for 10 minutes at 37°C.

Add stop solution.

Read the OD value within 15 minutes at 450nm.

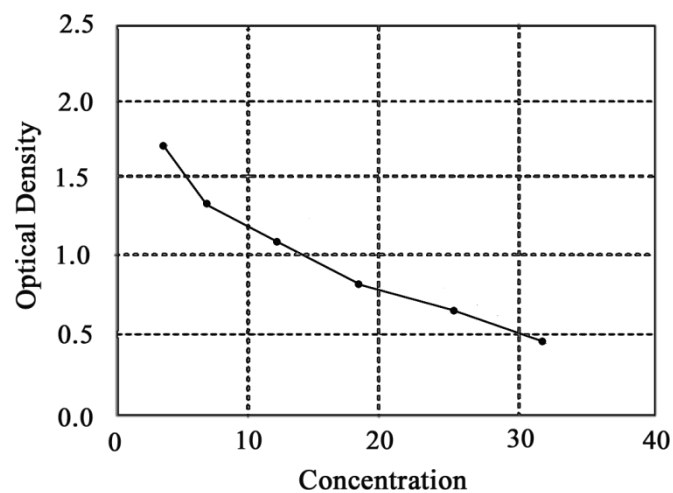
Calculation of Results

Average the duplicate readings for each standard, control, and sample. Create a standard curve by plotting the mean absorbance for each standard on the Y-axis against the target antigen concentration on the X-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the target antigen concentration on the X axis versus the O.D. of the standards on the Y axis and the best fit line can be determined by regression analysis. The linear equation ($X = Y + \text{Calibration Value}$) can be used to calculate the standard curve where X is the log of the concentration of the standard and Y is the OD value of the standard. If samples have been diluted (2-5 times is recommended), the concentration read from the standard curve must be multiplied by the dilution factor.

Concentration	Blank	S5	S4	S3	S2	S1	S0
OD value	VB	V5	V4	V3	V2	V1	V0
Calibration value	VB-VB	V5-VB	V4-VB	V3-VB	V2-VB	V1-VB	V0-VB

Typical Data

This standard curve is only for demonstration purposes. A standard curve should be generated with each assay.



Troubleshooting

Possible Case

High Background

- Improper washing
- Substrate was contaminated
- Non-specific binding of antibody
- Plate are not be sealing incompletely
- Incorrect incubation temperature
- Substrate exposed to light prior to use
- Contaminated wash buffer

Solution

- Increasing duration of soaking steps
- Replace. Substrate should be clean and avoid crossed contamination by using the sealer
- Replace another purified antibody or blocking buffer
- Make sure to follow the instruction strictly
- Incubate at room temperature
- Keep substrate in a dark place
- Use a clean buffers and sterile filter

Weak Signal

- Improper washing
- Incorrect incubation temperature
- Antibody are not enough
- Reagent are contaminated
- Pipette are not clean
- Increasing duration of soaking steps
- Incubate at room temperature
- Increase the concentration of the antibody
- Use new one
- Pipette should be clean

No Signal

- Reagent are contaminated
- Sample prepared incorrectly
- Antibody are not enough
- Wash buffer contains sodium azide
- HRP was not added
- Use new one
- Make sure the sample workable/dilution
- Increase the antibody concentration
- Use a new wash buffer and avoid sodium azide in it
- Add HRP according to the instruction

Poor Standard Curve

- Improper standard dilution
- Inccorect storage of reagents
- Incomplete washing of the wells
- Capture antibody did not bind to the plate
- Reconstitute standard according to the instruction
- Store the reagents in the ELISA kit according to the printed instruction before using them
- Make sure wells are washed adequately by filling the wells with wash buffer and all residual antibody solutions crossed well before washing.
- Replace a new ELISA plate

Data Template

	1	2	3	4	5	6	7	8	9	10	11	12
A	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>
B	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>
C	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>
D	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>
E	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>
F	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>
G	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>
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