BT LAB
Bioassay Technology Laboratory
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Human Transglutaminase 6 Antibody, IgG ELISAKIT

Cat.No: ED0106Hu

Qualitative ELISA Kit User Instruction

This product is for research use only, not for use in diagnosis procedures. It's highly recommended to read this instruction entirely before the use.

Storage: Store at -20°C for one year. Or store at 2-8°C for 6 months. If individual reagents are opened it is recommended that the kit be used within 1 month. Avoid repeated thaw cycles.

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/mean x 100

Intra-assay: CV< 10%

Inter-assay: CV< 12%

Intended Use

This kit is for the Qualitative detection of Human Transglutaminase 6 antibody IgA (also known as TGM6 Ab IgG) in serum, plasma, cell culture supernatants, cell lysates, tissue homogenates or other biological fluids.

Assay Principle

This kit is based on a Qualitative reverse phase enzyme immunoassay technique. The microtiter plate has been pre-coated with a target antigen. Positive/Negative Controls or samples are added to the wells and incubate. Antibodies in the samples bind to the antigen on the plate. Unbound antibody is washed away during a washing step. A Horseradish Peroxidase (HRP) conjugated detection antibody is then added and incubate. Unbound 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 OD of an unknown sample can then be compared to the OD of the positive and negative controls in order to determine the presence of TGM6 Ab IgG.

Reagent Provided

Components	Quantity
Pre-coated plate	12 wells * 8 detachable strips
Positive control	0.5ml × 1 vial
Negative control	0.5ml × 1 vial
HRP conjugated	6ml x1
Sample diluent	6ml x1
Substrate solution A	6ml x1
Substrate solution B	6ml x1
Stop solution	6ml x1
Wash buffer (25x)	20ml × 1 vial
Plate sealer	2 pics
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Material Required but Not Supplied

- 37°C incubator
- Precision pipette and disposable tip
- Deionized or distilled water
- Clean tubes
- Absorbent paper
- Automatic plate washer or 8-channel pipette
- 500ml beaker and suitable measuring cylinder

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.
- Pipette tips and plate sealer in hand should be clean and disposable to avoid cross-contamination.
- Do not allow wells to become dry during the assay procedure.
- 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. Collect the supernatant without sediment.

Plasma Collect plasma using EDTA or heparin as an anticoagulant. After mix 10-20 minutes, centrifuge samples for 20 minutes at 2000-3000 RPM. Collect the supernatant without sediment.

Cell culture supernatant Collect by sterile tubes. When detecting secrete components, centrifuge at 2000-3000 RPM for 20 minutes. Collect the supernatants. When detecting the components in the cell, use PBS (pH 7.2-7.4) to dilute cell suspension, 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 20 minutes. Collect the supernatant without sediment.

Tissue Rinse tissues in ice-cold PBS (pH 7.4) to remove excess blood thoroughly and weigh before homogenization. Mince tissues and homogenize them in PBS (tissue weight (g): PBS (mL) volume=1:9) with a glass homogenizer on ice. To further break down the cells, you can sonicate the suspension with an ultrasonic cell disrupter or subject it to freeze-thaw cycles. The homogenates are then centrifuged for 15 minutes at 12,000 RPM at 4°C to get the supernatant. Avoid freeze/thaw cycles.

Urine/Ascites/Cerebrospinal fluid Collect by sterile tube. Centrifuge at 2000-3000 RPM for 20 minutes. Collect the supernatant without sediment.

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 3 months. Avoid repeated freeze thaw cycles.
- Samples should be brought to room temperature before starting the assay.
- Samples containing NaN3 can't be tested as it inhibits the activity of Horse Radish Peroxidase (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.

Reagent Preparation

- All reagents should be brought to room temperature before use.
- **Wash Buffer 25x** Dilute 25x 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 should be 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 4°C for up to one month.
3. Set a blank well without any solution.
4. Add 50ul negative control to each of the negative control wells and 50ul positive control to each of the positive control wells. Add 40ul sample diluent and then add 10ul sample to the sample well, mix well.
5. 5.Cover with a plate sealer, and incubate for 30 minutes at 37°C.
6. Remove the sealer and wash the plate 5 times with wash buffer. Soak wells with 300ul wash buffer for 30 seconds to 1 minute for each wash. For automated washing, aspirate all wells and wash 5 times with wash buffer. Blot the plate onto paper towels or other absorbent material.
7. Add 50ul HRP to each well (except blank well). Cover with a plate sealer, and incubate for 30 minutes at 37°C.
8. Remove the sealer and wash as described above.
9. Add 50ul substrate solution A to each well and then add 50ul substrate solution B to each well. Mix well. Incubate plate covered with a new sealer for 10 minutes at 37°C in the dark.
10. Add 50ul stop solution to each well, the blue color will change into yellow immediately.
11. 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 controls and set a blank control with no solution.

Add 50ul positive control or negative control to each well.

Dilute 10ul sample with 40ul sample diluent and add this diluted sample to each well.

Incubate for 30 minutes at 37°C.

Aspirate and wash 5 times.

Add HRP to each well 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 50ul stop solution.

Read the OD value within 15 minutes at 450nm.

Calculation of Result

Average the reading from duplicate or triplicate samples. For calculation the valence of Human Transglutaminase 6 Antibody, IgG ELISAKIT, compare the sample well with control.

Quality Control

- The average $OD_{positive} \geq 1.00$
- The average $OD_{negative} \leq 0.10$

Results

- Cutoff Value = average Negative Control value + 0.15
- While OD_{sample} < Cut-off Value: Negative
- While OD_{sample} ≥Cut-off Value: Positive

Troubleshooting

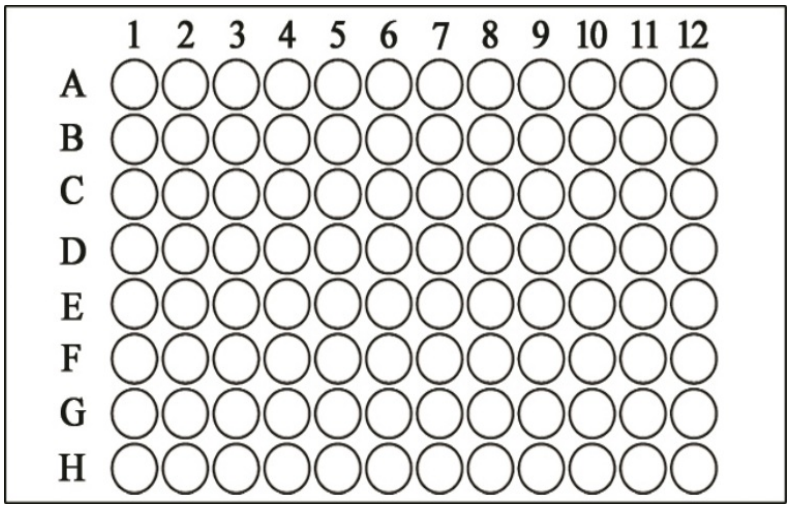
High Background possible case	Solution
Improper washing	Increasing duration of soaking steps
Incorrect incubation temperature	Incubate at 37°C
Incubation time too long	Reduce incubation time
Substrate exposed to light prior to use	Keep substrate in a dark place
Substrate was contaminated	Replace substrate. Substrate should be clean and avoid crossed contamination by using the sealer
Contaminated wash buffer	Use a clean buffers and sterile filter

Weak or No Signal possible case	Solution
A reagent or a step of the procedure omitted by mistake	Check protocol and follow steps carefully
Antibody are not enough	Increase the concentration of the antibody
Improper washing	Increasing duration of soaking steps
Reagent are contaminated	Use new one
Pipette are not clean	Pipette should be clean
HRP was not added	Add HRP according to the instruction
Sample contains sodium azide	Don't prepare samples with sodium azide
Wrong incubation time or temperature	Check and follow protocol. Place plates in an incubator during incubation periods (set to 37ºC).

Poor Precision possible case	Solution
Pipetting error	Dispense quickly and identically into the side of each well. Use calibrated pipettes.
Incomplete washing	Make sure wells are washed adequately by filling the wells with proper amount of wash buffer.
Unclean wells	Inspect wells and remove debris prior to use. Wipe the outer bottom of plate clean to remove any debris or fingerprints prior to reading.

Poor standard curve possible case	Solution
Incorrect preparation of standard	Reconstitute standard as suggested on data sheet.
Capture Antibody did not bind	Use BT LAB ELISA plate in the kit
Inefficient washing	Be sure wash apparatus is working properly (i.e. distributing even volumes into each well). Be sure wells are empty after aspiration, yet be sure to fill wells in a timely manner.
Pipetting error	Dispense quickly and identically into the side of each well. Use calibrated pipettes.
Incorrect storage of components	Store all components as recommended on data sheet. Do not allow reconstituted reagents to stay at room temperature for excess time.

Assay Principle



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