Version 17-03/25

User's Manual

Tartrazine (TZ) ELISA Kit

REF **DEIA25301**

Σ **96T**

RUO

This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

Creative Diagnostics

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PRODUCT INFORMATION

Intended Use

The kit is a competitive inhibition enzyme immunoassay technique for the in vitro quantitative measurement oftartrazine in serum, plasma, tissue homogenates, cell lysates, cell culture supernates and other biological fluids.

Principles of Testing

This assay employs the competitive inhibition enzyme immunoassay technique. A monoclonal antibody specificto tartrazine has been pre-coated onto a microplate. A competitive inhibition reaction is launched between biotinlabeled tartrazine and unlabeled tartrazine (Standards or samples) with the pre-coated antibody specific totartrazine. After incubation the unbound conjugate is washed off. Next, avidin conjugated to HorseradishPeroxidase (HRP) is added to each microplate well and incubated. The amount of bound HRP conjugate isreverse proportional to the concentration of tartrazine in the sample. After addition of the substrate solution, theintensity of color developed is reverse proportional to the concentration of tartrazine in the sample.

Reagents And Materials Provided

1. Pre-coated, ready to use 96-well strip plate, 1
2. Standard, 2
3. Detection Reagent A, 1×120μL
4. Detection Reagent B, 1×120μL
5. TMB Substrate, 1×9mL
6. Wash Buffer (30× concentrate), 1×20mL
7. Plate sealer for 96 wells, 4
8. Standard Diluent, 1×20mL
9. Assay Diluent A, 1×12mL
10. Assay Diluent B, 1×12mL
11. Stop Solution, 1×6mL

Materials Required But Not Supplied

1. Microplate reader with 450 ± 10nm filter.
2. Single or multi-channel pipettes with high precision and disposable tips.
3. Microcentrifuge Tubes.
4. Deionized or distilled water.
5. Absorbent paper for blotting the microplate.
6. Container for Wash Solution.

7. 0.01mol/L (or 1×) Phosphate Buffered Saline(PBS), pH7.0-7.2.

Storage

1. For unopened kit: All the reagents should be kept according to the labels on vials. The Standard, DetectionReagent A, Detection Reagent B and the 96-well strip plate should be stored at -20°C upon receipt whilethe others should be at 4°C.

2. For used kit: When the kit is used, the remaining reagents need to be stored according to the above storagecondition. Besides, please return the unused wells to the foil pouch containing the desiccant pack, andzip-seal the foil pouch.

Note:

It is highly recommended to use the remaining reagents within 1 month provided this is prior to the expiration dateof the kit. For the expiration date of the kit, please refer to the label on the kit box. All components are stable up tothe expiration date.

Specimen Collection And Preparation

Serum: Use a serum separator tube and allow samples to clot for two hours at room temperature or overnight at 4°C before centrifugation for 20 minutes at approximately 1,000×g. Assay freshly prepared serum immediately or store samples in aliquot at -20°C or -80°C for later use. Avoid repeated freeze/thaw cycles.

Plasma: Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge samples for 15 minutes at 1,000×g at 2-8°C within 30 minutes of collection. Remove plasma and assay immediately or store samples in aliquot at -20°C or -80°C for later use. Avoid repeated freeze/thaw cycles.

Tissue homogenates: The preparation of tissue homogenates will vary depending upon tissue type.

1. Tissues were rinsed in ice-cold PBS to remove excess blood thoroughly and weighed before homogenization.
2. Minced the tissues to small pieces and homogenized them in fresh lysis buffer (different lysis buffer needs to be chosen based on subcellular location of the target protein) (w:v = 1:20-1:50, e.g. 1mL lysis buffer is added in 20-50mg tissue sample) with a glass homogenizer on ice (Micro Tissue Grinders woks, too).
3. The resulting suspension was sonicated with an ultrasonic cell disrupter till the solution is clarified.
4. Then, the homogenates were centrifuged for 5 minutes at 10,000×g. Collect the supernates and assay immediately or aliquot and store at ≤-20°C.

Cell Lysates: Cells need to be lysed before assaying according to the following directions.

1. Adherent cells should be washed by cold PBS gently, and then detached with trypsin, and collected by centrifugation at 1,000×g for 5 minutes (suspension cells can be collected by centrifugation directly).
2. Wash cells three times in cold PBS.
3. Resuspend cells in fresh lysis buffer with concentration of 10^7 cells/mL. If it is necessary, the cells could be subjected to ultrasonication till the solution is clarified.
4. Centrifuge at 1,500×g for 10 minutes at 2-8°C to remove cellular debris. Assay immediately or aliquot and store at ≤-20°C.

Cell culture supernates and other biological fluids: Centrifuge samples for 20 minutes at 1,000×g. Collect

the supernates and assay immediately or store samples in aliquot at -20°C or -80°C for later use. Avoid repeatedfreeze/thaw cycles.

Note:

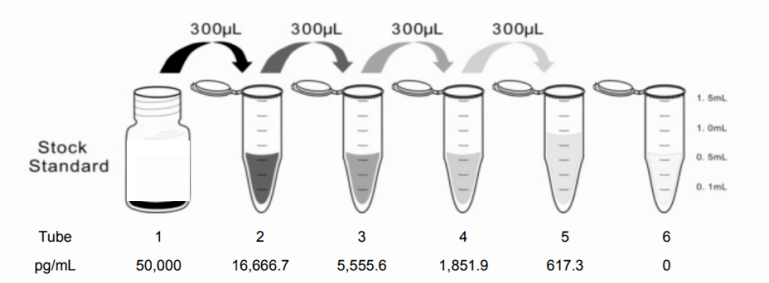
1. Samples to be used within 5 days may be stored at 4°C, otherwise samples must be stored at -20°C (≤1month) or -80°C (≤2 months) to avoid loss of bioactivity and contamination.
2. Sample hemolysis will influence the result, so hemolytic specimen should not be used.
3. When performing the assay, bring samples to room temperature.
4. It is highly recommended to use serum instead of plasma for the detection based on quantity of our in-house data.

[SAMPLE PREPARATION]

1. We are only responsible for the kit itself, but not for the samples consumed during the assay. The user shouldcalculate the possible amount of the samples used in the whole test. Please reserve sufficient samples inadvance.
2. Please predict the concentration before assaying. If values for these are not within the range of the standardcurve, users must determine the optimal sample dilutions for their particular experiments. Sample should bediluted by PBS.
3. If the samples are not indicated in the manual, a preliminary experiment to determine the validity of the kit is necessary.
4. Tissue or cell extraction samples prepared by chemical lysis buffer may cause unexpected ELISA results dueto the impacts from certain chemicals.
5. Due to the possibility of mismatching between antigen from other origin and antibody used in our kits (e.g.,antibody targets conformational epitope rather than linear epitope), some native or recombinant proteinsfrom other manufacturers may not be recognized by our products.
6. Influenced by the factors including cell viability, cell number or sampling time, samples from cell culturesupernates may not be detected by the kit.
7. Fresh samples without long time storage is recommended for the test. Otherwise, protein degradation and denaturalization may occur in those samples and finally lead to wrong results.

Reagent Preparation

1. Bring all kit components and samples to room temperature (18-25°C) before use. If the kit will not be used upin one time, please only take out strips and reagents for present experiment, and leave the remaining stripsand reagents in required condition.
2. Standard: Reconstitute the Standard with 0.5mL of Standard Diluent, kept for 10 minutes at room temperature, shake gently(not to foam). The concentration of the standard in the stock solution is 50,000pg/mL. Please prepare 5 tubes containing 0.6mL Standard Diluent and produce a triple dilution series according to the picture shown below. Mix each tube thoroughly before the next transfer. Set up 5 points of diluted standard such as 50,000pg/mL, 16,666.7pg/mL, 5,555.6pg/mL, 1,851.9pg/mL, 617.3pg/mL, and the last EP tubes with Standard Diluent is the blank as 0pg/mL.



3. Detection Reagent A and Detection Reagent B: Briefly spin or centrifuge the stock Detection A and Detection B before use. Dilute them to the working concentration 100-fold with Assay Diluent A and B, respectively.
4. Wash Solution: Dilute 20mL of Wash Solution concentrate (30×) with 580mL of deionized or distilled water to prepare 600mL of Wash Solution (1×).
5. TMB substrate: Aspirate the needed dosage of the solution with sterilized tips and do not dump the residual solution into the vial again.

Note:

1. Making serial dilution in the wells directly is not permitted.
2. Prepare standard within 15 minutes before assay. Please do not dissolve the reagents at 37oC directly.
3. Detection Reagent A and B are sticky solutions, therefore, slowly pipette them to reduce the volume errors.
4. Please carefully reconstitute Standards or working Detection Reagent A and B according to the instruction, and avoid foaming and mix gently until the crystals are completely dissolved. To minimize imprecision caused by pipetting, use small volumes and ensure that pipettors are calibrated. It is recommended to suck more than 10μL for one pipetting.
5. The reconstituted Standards, Detection Reagent A and Detection Reagent B can be used only once.
6. If crystals have formed in the Wash Solution concentrate (30×), warm to room temperature and mix gently until the crystals are completely dissolved.
7. Contaminated water or container for reagent preparation will influence the detection result.

Assay Procedure

1. Determine wells for diluted standard, blank and sample. Prepare 5 wells for standard points, 1 well for blank. Add 50μL each of dilutions of standard (read Reagent Preparation), blank and samples into the appropriate wells, respectively. And then add 50μL of Detection Reagent A to each well immediately. Shake the plate gently (using a microplate shaker is recommended). Cover with a Plate sealer. Incubate for 1 hour at 37°C. Detection Reagent A may appear cloudy. Warm to room temperature and mix gently until solution appears uniform.
2. Aspirate the solution and wash with 350μL of 1× Wash Solution to each well using a squirt bottle, multi-channel pipette, manifold dispenser or autowasher, and let it sit for 1-2 minutes. Remove the remaining liquid from all wells completely by snapping the plate onto absorbent paper. Repeat 3 times. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against absorbent paper.
3. Add 100μL of Detection Reagent B working solution to each well. Incubate for 30 minutes at 37°C after

covering it with the Plate sealer.

4. Repeat the aspiration/wash process for total 5 times as conducted in step 2.
5. Add 90μL of Substrate Solution to each well. Cover with a new Plate sealer. Incubate for 10 - 20 minutes at 37°C (Don't exceed 30 minutes). Protect from light. The liquid will turn blue by the addition of Substrate Solution.
6. Add 50μL of Stop Solution to each well. The liquid will turn yellow by the addition of Stop solution. Mix the liquid by tapping the side of the plate. If color change does not appear uniform, gently tap the plate to ensure thorough mixing.
7. Remove any drop of water and fingerprint on the bottom of the plate and confirm there is no bubble on the surface of the liquid. Then, run the microplate reader and conduct measurement at 450nm immediately.

Note:

1. Assay preparation: Keep appropriate numbers of wells for each experiment and remove extra wells frommicroplate. Rest wells should be resealed and stored at -20°C.
2. Samples or reagents addition:Please use the freshly prepared Standard. Please carefully add samplesto wells and mix gently to avoid foaming. Do not touch the well wall. For each step in the procedure, totaldispensing time for addition of reagents or samples to the assay plate should not exceed 10 minutes. This will ensure equal elapsed time for each pipetting step, without interruption. Duplication of all standards andspecimens, although not required, is recommended. To avoid cross-contamination, change pipette tipsbetween additions of standards, samples, and reagents. Also, use separated reservoirs for each reagent.
3. Incubation: To ensure accurate results, proper adhesion of plate sealers during incubation steps isnecessary. Do not allow wells to sit uncovered for extended periods between incubation steps. Oncereagents are added to the well strips, DO NOT let the strips DRY at any time during the assay. Incubationtime and temperature must be controlled.
4. Washing: The wash procedure is critical. Complete removal of liquid at each step is essential for goodperformance. After the last wash, remove any remaining Wash Solution by aspirating or decanting andremove any drop of water and fingerprint on the bottom of the plate. Insufficient washing will result in poorprecision and false elevated absorbance reading.
5. Controlling of reaction time: Observe the change of color after adding TMB Substrate (e.g. observation once every 10 minutes), if the color is too deep, add Stop Solution in advance to avoid excessively strong reaction which will result in inaccurate absorbance reading.
6. TMB Substrate is easily contaminated. Please protect it from light.
7. The environment humidity which is less than 60% might have some effects on the final performance,therefore, a humidifier is recommended to be used at that condition.

Calculation

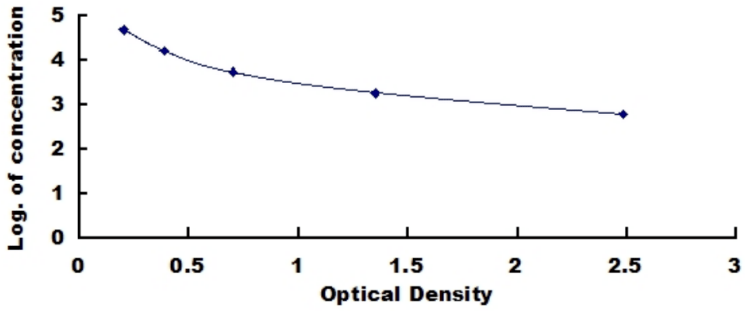
This assay employs the competitive inhibition enzyme immunoassay technique, so there is an inverse correlationbetween tartrazine concentration in the sample and the assay signal intensity.

Average the duplicate readings for each standard, control, and samples. Create a standard curve on log-log orsemi-log graph paper, with the log of tartrazine concentration on the y-axis and absorbance on the x-axis. Drawthe best fit straight line through the standard points and it can be determined by regression analysis. Using someplot software, for instance, curve expert 1.30, is also recommended. If samples have been

diluted, theconcentration read from the standard curve must be multiplied by the dilution factor.

Typical Standard Curve

In order to make the calculation easier, we plot the O.D. value of the standard (X-axis) against the log ofconcentration of the standard (Y-axis), although concentration is the independent variable and O.D. value is thedependent variable. The O.D. values of the standard curve may vary according to the conditions of assayperformance (e.g. operator, pipetting technique, washing technique or temperature effects). Typical standardcurve below is provided for reference only.



Performance Characteristics

The stability of ELISA kit is determined by the loss rate of activity. The loss rate of this kit is less than 5% withinthe expiration date under appropriate storage condition.

To minimize extra influence on the performance, operation procedures and lab conditions, especially roomtemperature, air humidity, incubator temperature should be strictly controlled. It is also strongly suggested that thewhole assay is performed by the same operator from the beginning to the end.

Precision

Intra-assay Precision (Precision within an assay): 3 samples with low, middle and high level tartrazine were tested 20 times on one plate, respectively.

Inter-assay Precision (Precision between assays): 3 samples with low, middle and high level tartrazine were tested on 3 different plates, 8 replicates in each plate. CV(%) = SD/mean × 100

Intra-Assay: CV<10%

Inter-Assay: CV<12%

Detection Range

617.3-50,000pg/mL. The standard curve concentrations used for the ELISA's were 50,000pg/mL, 16,666.7pg/mL, 5,555.6pg/mL, 1,851.9pg/mL, 617.3pg/mL.

Sensitivity

The minimum detectable dose of tartrazine is typically less than 284.4pg/mL.

The sensitivity of this assay, or Lower Limit of Detection (LLD) was defined as the lowest protein

concentrationthat could be differentiated from zero. It was determined by subtracting two standard deviations to the meanoptical density value of twenty zero standard replicates and calculating the corresponding concentration.

Specificity

This assay has high sensitivity and excellent specificity for detection of tartrazine. No significant cross-reactivity or interference between tartrazine and analogues was observed.

Note:

Limited by current skills and knowledge, it is impossible for us to complete the cross- reactivity detection between tartrazine and all the analogues, therefore, cross reaction may still exist.

Linearity

The linearity of the kit was assayed by testing samples spiked with appropriate concentration of tartrazine andtheir serial dilutions. The results were demonstrated by the percentage of calculated concentration to theexpected.

Sample	1：2	1：4	1：8	1：16
serum(n=5)	89-101%	85-95%	79-98%	84-104%
EDTA plasma(n=5)	96-108%	86-99%	90-102%	78-92%
heparin plasma(n=5)	80-93%	81-97%	88-104%	87-99%

Recovery

Matrices listed below were spiked with certain level of tartrazine and the recovery rates were calculated bycomparing the measured value to the expected amount of tartrazine in samples.

Matrix	Recovery range (%)	Average(%)
serum(n=5)	81-94	87
EDTA plasma(n=5)	85-103	95
heparin plasma(n=5)	79-97	90