



KAMIYA BIOMEDICAL COMPANY

Human Tyrosinase (Tyr) ELISA

**For the quantitative determination of human Tyr in
biological samples**

Cat. No. KT-50955

For Research Use Only. Not for use in diagnostic procedures.

Product Information
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INTENDED USE

This ELISA kit is a sandwich enzyme immunoassay for the *in vitro* quantitative measurement of human Tyr in biological samples. For research use only. Not for use in diagnostic procedures.

PRINCIPLE

This Tyr assay applies a technique called a quantitative sandwich immunoassay. The microtiter plate provided in the kit has been pre-coated with a monoclonal antibody specific for Tyr. Calibrators or samples are then added to the microtiter plate wells and if Tyr is present it will bind to the antibody coated plate. In order to quantitatively determine the amount of Tyr present in the each well a preparation of horseradish peroxidase (HRP) conjugated polyclonal antibody, specific for Tyr, is added to each well to “sandwich” the immobilized Tyr on the plate. The microtiter plate undergoes incubation, and then the wells are thoroughly washed to remove all unbound components. Next, substrate solutions A and B are added to each well. The enzyme and substrate are allowed to react over a short incubation period. Only those wells that contain Tyr and enzyme-conjugate antibody will exhibit a change in color. The enzyme-substrate reaction is terminated by the addition of a sulphuric acid solution and the color change is measured spectrophotometrically at a wavelength of 450 nm.

COMPONENTS

Reagents	Quantity
Pre-coated 96-well plate	1
Calibrator 1 (0 ng/mL)	1
Calibrator 2 (5 ng/mL)	1
Calibrator 3 (10 ng/mL)	1
Calibrator 4 (25 ng/mL)	1
Calibrator 5 (50 ng/mL)	1
Calibrator 6 (100 ng/mL)	1
Enzyme Conjugate	1 x 10 mL
Lysis Buffer Solution	1 x 10 mL
Substrate A	1 x 6 mL
Substrate B	1 x 6 mL
Stop Solution	1 x 6 mL
Wash Buffer (100X concentrate)	1 x 10 mL

STORAGE

All reagents should be stored at 4°C upon receipt. For expiration date refer to kit label.

SAMPLE COLLECTION AND STORAGE

Serum

Use a serum separator tube and allow samples to clot for 30 minutes before centrifugation for 15 minutes at approximately 1,000 x g. Remove serum and assay immediately or store samples in aliquots 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 x g at 4°C within 30 minutes of collection. Assay immediately or store samples in aliquots at -20°C or -80°C for later use. Avoid repeated freeze/thaw cycles.

Tissue Homogenates

Use between 0.3 and 0.5 g of tissue. Evenly cut the tissue into small pieces. Add 500 µL of PBS (1:10-1:20) or saline to wash the tissue. Wash 1-2 times as necessary. Centrifuge at 5,000 RPM for 10 minutes and remove the supernate. Assay immediately or aliquot and store samples at -20°C.

Cell Culture Supernates and Other Biological Fluids

Remove particulates by centrifugation and assay immediately or aliquot and store samples at -20°C or -80°C. Avoid repeated freeze-thaw cycles.



Note:

1. Samples to be used within 7 days may be stored at 4°C, otherwise samples must be stored at -20°C (≤1 month) or -80°C (≤2 months) to avoid loss of bioactivity and contamination.
2. When performing the assay, slowly bring samples to room temperature.
3. Sample hemolysis will influence the result, so hemolytic specimen cannot be detected.
4. Do not use heat treated specimens.

MATERIALS REQUIRED BUT NOT SUPPLIED

1. Microplate reader with 450 nm filter.
2. Precision pipettes to deliver 1-2 mL volumes.
3. Adjustable 10-100 mL pipettes for reagent preparation.
4. 100 mL and 1 L graduated cylinders.
5. Calibrated adjustable precision pipettes with disposable tips (multi-channel is desirable for large assays).
6. 37°C incubator.
7. Absorbent paper.
8. Distilled or deionized water
9. Data analysis tools such as graphing software, or graph paper (linear, log-log, semi-log, or log-logit as desired).
10. Tubes to prepare Calibrators or sample dilutions.

Precautions

1. Do not substitute reagents from one kit lot to another. Calibrators, conjugate, and microtiter plates are matched for optimal performance. Use only reagents supplied by manufacturer.
2. Allow kit reagents and materials to reach room temperature (20-25°C) before use. Do not use water baths to thaw samples or reagents.
3. Do not use components beyond their expiration date.
4. Use only deionized or distilled water to dilute reagents.
5. Do not remove microtiter plate from the storage bag until needed. Unused strips should be stored at 4°C in their pouch with the desiccant provided.
6. Use fresh disposable pipette tips for each transfer to avoid contamination.
7. Do not mix acid and sodium hypochlorite solutions.
8. Samples should be handled as potentially hazardous and capable of transmitting disease. Disposable gloves must be worn during the assay procedure, since no known test method can offer complete assurance that products derived from humans will not transmit infectious agents. Therefore,

all samples should be considered potentially infectious and good laboratory practices should be followed.

9. All samples should be disposed of in a manner that will inactivate viruses.
10. Solid waste: Autoclave 60 min at 121°C.
11. Liquid waste: Add sodium hypochlorite to a final concentration of 1.0%. The waste should be allowed to stand for a minimum of 30 minutes to inactivate the viruses before disposal.
12. Substrate solution is easily contaminated. If bluish prior to use, do not use.
13. Substrate B contains 20% acetone, keep this reagent away from sources of heat or flame.
14. Remove all kit reagents from refrigerator and allow them to reach room temperature (20-25°C).

ASSAY PROCEDURE

1. Prepare all calibrators before starting assay procedure (see Reagent Preparation). It is recommended that all calibrators and samples be added in duplicate to the microtiter plate.
2. First, secure the desired number of coated wells in the holder, then add 50 µL of calibrators and samples to the appropriate well of the antibody coated microtiter plate.
3. Add 100 µL of conjugate to each well. Mix well. Complete mixing in this step is important. Cover and incubate for 1 hour at 37°C.
4. Prepare substrate solution no more than 15 minutes before end of incubation (see Reagent Preparation).
5. Wash the microtiter plate using one of the specified methods indicated below:
6. Manual Washing: Remove incubation mixture by aspiration contents of the plate into a sink or proper waste container. Using a squirt bottle, fill each well completely with distilled or de-ionized water, then aspirate contents of the plate into a sink or proper waste container. Repeat this procedure four more times for a total of five washes. After final wash, invert plate, and blot dry by hitting plate onto absorbent paper or paper towels until no moisture appears. Note: Hold the sides of the plate frame firmly when washing the plate to assure that all strips remain securely in frame.
7. Automated Washing: Aspirate all wells, then wash plate five times using distilled or de-ionized water. Always adjust your washer to aspirate as much liquid as possible and set fill volume to 350 µL/well/wash (range: 350-400 µL). After final wash, invert plate, and blot dry by hitting plate onto absorbent paper and paper towels until no moisture appears. It is recommended that the washer be set for a soaking time of 15-30 seconds or shaking time of 5 seconds between washes.
8. Add 50 µL each of substrate A and B to each well. Cover and incubate for 15 minutes at 20-25°C.
9. Add 50 µL stop solution to each well. Mix well.
10. Read the optical density (OD) at 450 nm using a microtiter plate reader within 30 minutes.

CALCULATION OF RESULTS

1. The calibration curve is used to determine the amount in an unknown sample. The calibration curve is generated by plotting the average OD (450 nm) obtained from each of the six calibrator concentrations on the vertical (Y) axis versus the corresponding concentration on the horizontal (X) axis.
2. First, calculate the mean OD value for each calibrator and sample. Subtract the mean value of the zero calibrator from all OD values before interpreting the result. Construct the calibration curve using graph paper or statistical software.
3. To determine the amount in each sample, first locate the value on the Y-axis and extend a horizontal line to the calibration curve. At the point of intersection, draw a vertical line to the X-axis and read the corresponding concentration.
4. Any variations in the operator, pipetting and washing technique, incubation time, temperature, or kit age can cause variation in the result. All users should obtain their own calibration curve.
5. The sensitivity of this assay is 0.01 ng/mL.

REAGENT PREPARATION

All reagents must be allowed to reach room temperature before use. Additional information for individual reagents can be found on vial labels.

Dispense 10 µL of lysis buffer solution into 100 µL samples, mix and stand for one hour. The proportion of Lysis Buffer Solution and samples should be 1:10.

To prepare the Wash Solution dilute 10 mL of Wash Solution concentrate (100x) with 990 mL of deionized or distilled water to prepare 1000 mL of Wash Solution (1x).

**Note:**

1. This kit contains materials with small quantities of sodium azide. Sodium azide reacts with lead and copper plumbing to form explosive metal azides. Upon disposal, flush drain with a large volume of water to prevent azide accumulation. Avoid ingestion and contact with eyes, skin, and mucous membranes. In case of contact, rinse affected area with plenty of water. Observe all federal, state, and local regulations for disposal.
2. All blood components and biological materials should be handled as potentially hazardous. Follow universal precautions as established by the Centers for Disease Control and Prevention and by the Occupational Safety and Health Administration when handling and disposing of infectious agents.

IMPORTANT NOTES

1. When not in use, kit components should be refrigerated. All reagents should be warmed to room temperature before use.
2. Microtiter plates should be allowed to come to room temperature before opening the foil bags. Once the desired number of strips has been removed, immediately reseal the bag and store at 4°C to maintain plate integrity.
3. Samples should be collected in pyrogen/endotoxin free tubes.
4. Samples should be frozen if not analyzed shortly after collection. Avoid multiple freeze-thaw cycles of frozen samples. Thaw completely and mix well prior to analysis.
5. When possible, avoid use of badly hemolyzed or lipemic sera. If large amounts of particulate matter are present, centrifuge or filter prior to analysis.
6. It is recommended that all calibrators, controls, and samples be run in duplicate.
7. When pipetting reagents maintain a consistent order from well-to-well. This ensures equal incubation time for all wells.
8. Cover or cap all reagents when not in use.
9. Do not mix or interchange different reagent lots from various kit lots.
10. Do not use reagents after the kit expiration date.
11. Read absorbances within 2 hours of assay completion.
12. The provided protocols should be run with every assay. If control values fall outside pre-established ranges, the accuracy of the assay is suspect.
13. All residual wash liquid must be drained from the wells by efficient aspiration or by decantation followed by tapping the plate forcefully on absorbent paper. Never insert absorbent paper directly into wells.
14. Because stabilized Chromogen is light sensitive, avoid prolonged exposure to light. Also avoid contact between stabilized Chromogen and metal, or color may develop.
15. Incomplete washing will adversely affect the test outcome. All washing must be performed with wash buffer provided.
16. Washing can be performed manually as follows: completely aspirate the liquid from all wells by gently lowering Tyr aspiration tip (aspiration device) into the bottom of each well. Take care not to scratch the inside of the well.
17. After aspiration, fill the wells with at least 0.35 mL of diluted wash solution. Let soak for 15-30 seconds, then aspirate the liquid. Repeat as directed under Assay Procedure. After the washing procedure, the plate is inverted and tapped dry on absorbent tissue.
18. Alternatively, the wash solution may be put into a squirt bottle. If a squirt bottle is used, flood the plate with wash buffer, completely filling all wells. After the washing procedure, the plate is inverted and tapped dry on absorbent tissue.
19. If using an automated washer, the operating instructions for washing equipment should be carefully followed.
20. Assay Procedure Preliminary notes: Do not mix reagents from different lots. It is recommended that assays be performed in duplicate. Calibrators and samples must be assayed at the same time. Avoid exposing the substrate to direct sunlight.

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