Rat E2(Estradiol) ELISA Kit

Catalog #:KE1818

Detection and Quantification of Rat E2(Estradiol) in Serum, Plasma, Biological Fluids.

Please read the provided manual as suggested experimental protocols may have changed.

Research Purposes Only. Not Intended for Diagnostic or Clinical Procedures.

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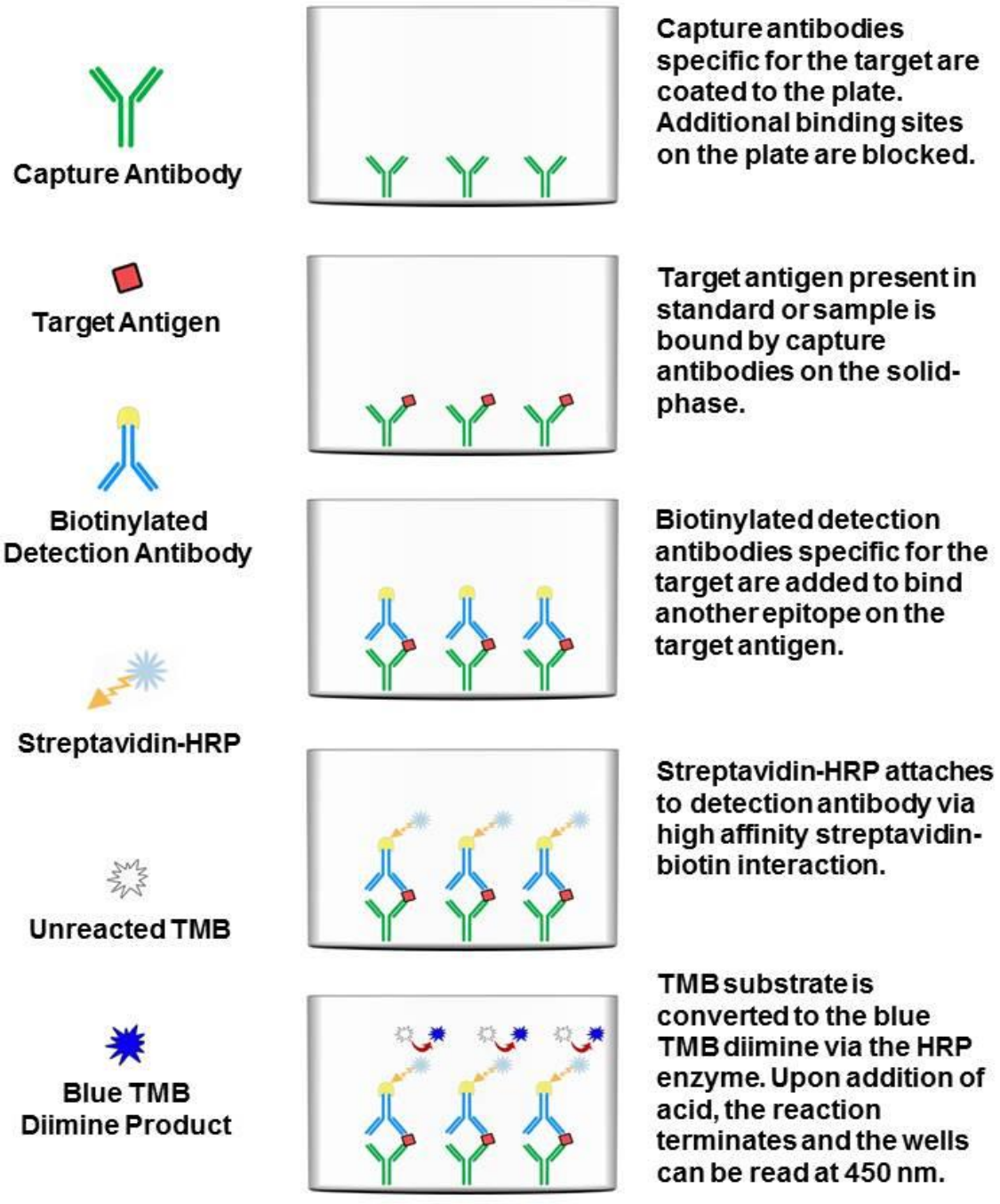
ASSAY PRINCIPLES

The Rat E2(Estradiol) ELISA Kit contains the components necessary for quantitative determination of natural or recombinant Rat E2(Estradiol) concentrations within any experimental sample including cell lysates, serum and plasma. This ELISA kit uses the Competitive-ELISA principle. The micro ELISA plate provided in this kit has been pre-coated with Rat E2. Samples (or Standards) and Horseradish Peroxidase (HRP) linked antibody specific for Rat E2 are added to the micro ELISA plate wells. Rat E2 in samples (or standards) competes with a fixed amount of E2 on the solid phase supporter for sites on the HRP linked detection antibody specific to E2. Excess conjugate and unbound sample or standard are washed from the plate. The substrate solution is added to each well. The enzyme-substrate reaction is terminated by the addition of stop solution and the color turns yellow. The optical density (OD) is measured spectrophotometrically at a wavelength of 450±2 nm. The concentration of Rat E2 in the samples is then determined by comparing the OD of the samples to the standard curve.

ASSAY FORMAT



ASSAY RESTRICTIONS

- This ELISA kit is intended for research purposes only, NOT diagnostic or clinical procedures of any kind.
- Materials included in this kit should NOT be used past the expiration date on the kit label.
- Reagents or substrates included in this kit should NOT be mixed or substituted with reagents or substrates from any other kits.
- Variations in pipetting technique, washing technique, operator laboratory technique, kit age, incubation time or temperature may cause differences in binding affinity of the materials provided.
- The assay is designed to eliminate interference and background by other cellular macromolecules or factors present within any biological samples. However, the possibility of background noise cannot be fully excluded until all factors have been tested using the assay kit.

MATERIALS INCLUDED

Component	Quantity Per Plate
Microstrips Coated w/ Capture Antibody	12 x 8-WellMicrostrips
Protein Standard	200pg/tube*2
HRP-Detection Ab(100×)	60μL
HRP-Detection Ab Diluent	14ml
Standard & Sample Diluent	20ml
Wash Buffer (25x)	30ml
Substrate Reagent(TMB)	10ml
Stop Solution	10 ml
Adhesive Plate Sealers	5 Sheets
Technical Manual	1 Manual

ADDITIONAL MATERIALS REQUIRED

The following materials and/or equipment are NOT provided in this kit but are necessary to successfully conduct the experiment:

- Microplate reader able to measure absorbance at 450 nm (with correction wavelength set to 540 nm or 570 nm)
- Micropipettes with capability of measuring volumes ranging from 1 µl to 1 ml
- Deionized or sterile water
- Squirt bottle, manifold dispenser, multichannel pipette reservoir or automated microplate washer
- Graph paper or computer software capable of generating or displaying logarithmic functions
- Absorbent paper or vacuum aspirator
- Test tubes or microfuge tubes capable of storing ≥1 ml
- Bench-top centrifuge (optional)
- Bench-top vortex (optional)
- Orbital shaker (optional)

HEALTH AND SAFETY PRECAUTIONS

- Reagents provided in this kit may be harmful if ingested, inhaled or absorbed through the skin. Please carefully review the MSDS for each reagent before conducting the experiment.
- Stop Solution contains 2 N Sulfuric Acid (H_2SO_4) and is an extremely corrosive agent. Please wear proper eye, hand and face protection when handling this material. When the experiment is finished, be sure to rinse the plate with copious amounts of running water to dilute the Stop Solution prior to disposing the plate.

STORAGE INFORMATION

Note: If used frequently, reagents may be stored at 4°C.

Unopened Kits: Store at 4°C for 6 months.

Component	Storage Time	Storage Information
Microstrips Coated w/ Capture Antibody	6 Months	4°C
HRP Detection Ab		
Wash Buffer (25x)		
Assay Diluent		
Substrate Reagent(TMB)		
Stop Solution		
Protein Standard	Lyophilized: 6 Months Reconstituted: 1 Month	-4°C
Adhesive Plate Sealers	-	-
Technical Manual	-	-

SAMPLE PREPARATION AND STORAGE

If samples are to be used within 24 hours, aliquot and store at 4°C. If samples are to be used over a long period of time, aliquot and store between -20°C and -80°C, depending on the duration of storage.

Note: Samples containing a visible precipitate or pellet must be clarified prior to use in the assay.

Caution: Avoid repeated freeze/thaw cycles to prevent loss of biological activity of proteins in experimental samples.

Cell Lysate and Supernatants

Remove large cell components via centrifugation and perform the assay. Cell lysates and supernatants require a dilution using Assay Diluent. A serial dilution may be performed to determine a suitable dilution factor for the sample. For future use of the sample, follow the sample storage guidelines stated above.

Serum

Allow samples to clot in a serum separator tube (SST) for 30 minutes. After sufficient clotting, centrifuge at 1000 x g for 15 minutes and remove serum from SST in preparation for the assay. Serum samples may require a dilution using Assay Diluent. For future use of the sample, follow the storage guidelines above.

Plasma

Use heparin, citrate or EDTA as an anticoagulant to gather plasma from original biological sample. After collection of the plasma, centrifuge for 15 minutes at 1000 x g. This step must be performed within 30 minutes of plasma collection. Plasma samples may require a dilution using Assay Diluent. Afterwards, perform the assay or for future use of the sample, follow the storage guidelines stated above.

IMMUNOASSAY PROTOCOL

Note: If possible, all incubation steps should be performed on an orbital shaker to equilibrate solutions when added to the microplate wells. Also, all provided solutions should be at ambient temperature prior to use.

Note: Avoid adding solutions into wells at an angle, always keep pipette tip perpendicular to plate bottom.

1 Reconstitution of Provided Materials

Dilute the 25x Wash Buffer in ddH_2O for 1x Wash Buffer.

2 Addition of Known Standard and Unknown Sample to Immunoassay

The E2 ELISA Kit allows for the detection and quantification of endogenous levels of natural and/or recombinant E2 within the range of 3.13-200pg/mL.

Add 50 µl of each proptein standard or samples into the wells of a specified row or column of the 96-well microtiter plate

3 Addition of HRP-Detection Ab to Capture Antibody-Bound Samples

1. Add 50 µl of ***HRP-Detection Ab*** into each well, Seal the microplate air-tight using one of the microplate adhesive seals provided in this kit or Parafilm if readily available.
2. Seal the plate and incubate at 37° C for 60min.

Remove the detection solution out of the microplate wells by either vacuum-based aspirator or paper towel blotting. Perform 4 consecutive wash steps with gentle shaking between each wash, using 350µl washing buffer per well, 1-2minutes for each wash.

4 Application of Liquid Substrate for Colorimetric Reaction

1. After the 4th wash step, add 90 µl of ***Substrate Reagent(TMB)*** solution into each well and incubate at room temperature for color development. The microplate should be kept out of direct light by either covering with an opaque object or putting it into a dark room. Closely monitor the color development as some wells may turn blue

very quickly depending on analyte and/or detection antibody-HRP concentrations.

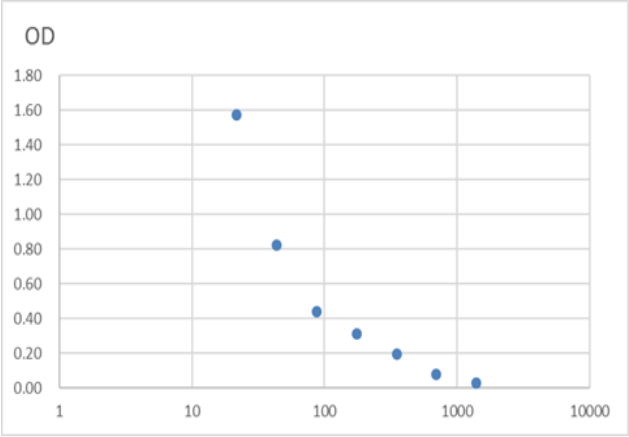
2. Once the blue color has ceased to develop further(15-30min), immediately add 50 µl of ***Stop Solution*** to each well being used. The color in the wells should immediately change from blue to yellow.
3. The microplate is now ready to be read by a microplate reader. Within 30 minutes of adding the ***Stop Solution***, determine the optical density (absorbance) of each well by reading the plate with the microplate reader set to 450 nm.

Generation of Standard Curve and Interpretation of Data

1. Average the duplicate or triplicate readings for each standard, control and sample and subtract the average zero standard optical density.
2. Generate a standard curve by using Microsoft Excel or other computer software capable of establishing a 4-Parameter Logistic (4-PL) curve fit. If using Excel or an alternative graphing tool, plot the average optical density values in absorbance units (y-axis) against the known standard concentrations in pg/ml (x-axis). **Note:** Only use the values in which a noticeable gradient can be established. Afterwards, generate a best fit curve or “trend-line” through the plotted points via regression analysis. **Note:** Shown on the next page is an example of typical data produced by analysis of the standard sample.

TYPICAL DATA

As the OD values of the standard curve may vary according to the conditions of the actual assay performance (e.g. operator, pipetting technique, washing technique or temperature effects), the operator should establish a standard curve for each test. Typical standard curve and data is provided below for reference only.



SENSITIVITY

The Rat E2(Estradiol) ELISA Kit allows for the detection and quantification of endogenous levels of natural and/or recombinant Rat E2(Estradiol) within the range of 3.13-200 pg/mL

TECHNICAL SUPPORT

For troubleshooting, information or assistance, please go online to www.immunoway.com.

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NOTES

Over 3,000 Assay Kits including Competive, Cell-Based and Transcription Factor ELISA Kits

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