Rat Phosphorylated Smad 3 (pSmad3) ELISA Kit

Item NO.	Product Name
KTE101158	Rat Phosphorylated Smad 3 (pSmad3) ELISA Kit

ATTENTION

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INTRODUCTION

Assay principle

Rat Phosphorylated Smad 3 (pSmad3) ELISA Kit employs a two-site sandwich ELISA to quantitate pSmad3 in samples. An antibody specific for Rat pSmad3 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any pSmad3 present is bound by the immobilized antibody. After removing any unbound substances, HRP-Conjugated pSmad3 detection antibody is added to the wells. Following a wash to remove any unbound HRP reagent, a Chromogen solution is added to the wells and color develops in proportion to the amount of pSmad3 bound in the initial step. The color development is stopped and the intensity of the color is measured.

Characteristics

- This Kit allows for the determination of pSmad3 concentrations in Rat serum, cell culture supernates and other biological fluids.
- Detection range: 0.15 ng/mL - 2.4 ng/mL.
- The minimum detectable dose (MDD) of Rat pSmad3 is typically less than 0.01 ng/mL.
- Four samples of known concentration were tested twenty times on one plate to assess intra-assay precision. The CV (%)＜9%.
- Three samples of known concentration were tested in twenty separate assays to assess inter-assay precision. Assays were performed by at least three technicians using two lots of components. The CV (%)＜11%.
- To assess linearity of the assay, samples containing and/or spiked with high concentrations of Rat pSmad3 were diluted with the appropriate calibrator diluent to produce samples with values within the dynamic range of the assay. Linear regression analysis of samples versus the expected concentration yielded a correlation coefficient of 0.99.
- Rat Phosphorylated Smad 3 (pSmad3) ELISA Kit has high sensitivity and excellent specificity for detection of Rat pSmad3. No significant cross-reactivity or interference between Rat pSmad3 and analogues was observed.

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PRODUCT INFORMATION

Materials supplied & Storage conditions

Store kit reagents at 2-8 °C for 6 months. Immediately after use remaining reagents should be returned to cold storage at 4 °C.

components	48T	96T	Storage conditions
Rat pSmad3 microplate	48 wells	96 wells	2-8 °C[1]
Rat pSmad3 standard	0.5 mL	0.5 mL	2-8 °C
HRP-Conjugated Rat pSmad3 detection antibody	3 mL	6 mL	2-8 °C
Standard diluent	1.5 mL	1.5 mL	2-8 °C
Sample diluent	3 mL	6 mL	2-8 °C
Chromogen solution A	3 mL	6 mL	2-8 °C
Chromogen solution B	3 mL	6 mL	2-8 °C
Stop solution	3 mL	6 mL	2-8 °C
Wash buffer	20 mL (20×)	20 mL (30×)	2-8 °C
Plate covers	1	2	RT
Booklet	1	1	RT

1 Return unused wells to the foil pouch containing the desiccant pack. Reseal along entire edge of the zip-seal. May be stored for up to 1 month at 2-8 °C.

Other supplies required

- 37 °C incubator.
- Standard microplate reader capable of measuring absorbance at 450 nm.
- Precision pipettes, disposable pipette tips and Absorbent paper.
- Distilled or deionized water.

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ASSAY PROTOCOL

Sample collection & storage

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Tissue homogenates - For general information, hemolysis blood may affect the result, so you should rinse the tissues with ice-cold PBS (0.01M, pH=7.4) to remove excess blood thoroughly. Tissue pieces should be weighed and then minced to small pieces which will be homogenized in PBS with a glass homogenizer on ice. (The volume depends on the weight of the tissue, 9mL PBS would be appropriate to 1 gram tissue pieces. Some protease inhibitor is recommended to add into the PBS.) To further break the cells, you can sonicate the suspension with an ultrasonic cell disrupter or subject it to freeze-thaw cycles. The homogenates are then centrifugated for 5 minutes at 5000×g to collect the supernate.

Cell culture supernatants and other biological fluids - Centrifuge samples for 20 minutes at 1000×g. Remove particulates and assay immediately or store samples in aliquot at -20 °C or -80 °C for later use. Avoid repeated freeze/thaw cycles.

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 1000×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 1000×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.
Note: Samples should be centrifugated adequately and no hemolysis or granule was allowed.

Reagent preparation

Bring all reagents to room temperature before use. If crystals were formed in the Buffer Concentrates, warm them gently until they completely dissolved.

Wash buffer - Dilute with Distilled or deionized water 1:20 (48T) /1:30 (96T).

Standard - Pipette 150 µL of Standard Diluent into each tube. Use the stock solution to produce a 2-fold dilution series (below). Mix each tube thoroughly before the next transfer. The undiluted Standard serves as the high standard. Standard Diluent serves as the zero standard.

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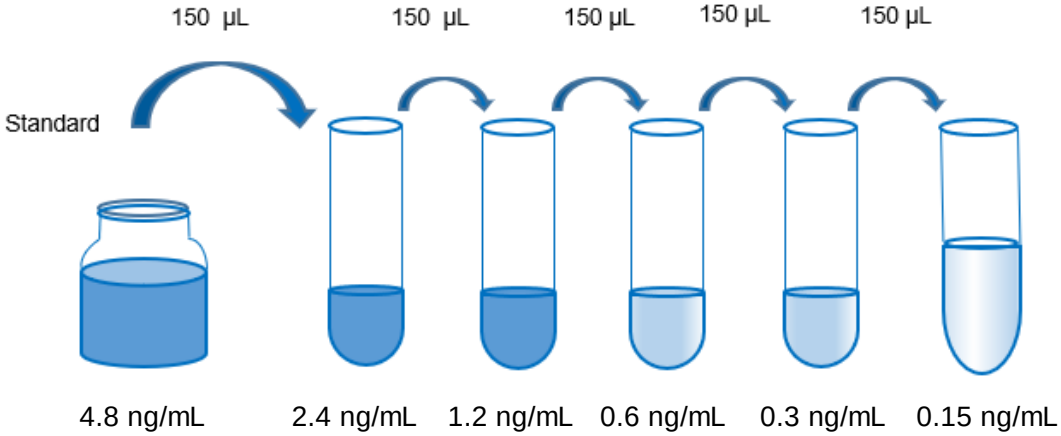

Note: If samples generate values higher than the highest standard, please dilute the samples with Sample Diluent and repeat the assay.

Assay procedure

1. Prepare all reagents before starting assay procedure. It is recommended that all Standards and Samples be added in duplicate to the microplate.
2. Add standard: Set Standard wells, testing sample wells. Add diluted standard 50 μL to standard well.
3. Add Sample: Add sample diluent 40 μL to testing sample well. Then add sample 10 μL to testing sample well, Blank well doesn't add anything.
4. Cover with a plate cover and incubate for 45 minutes at 37 °C.
5. Aspirate each well and wash, repeating the process four times for a total of five washes, 1-3 minutes per time. Wash by filling each well with Wash buffer (250 μL) using a squirt bottle, manifold dispenser or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
6. Add HRP-Conjugated detection antibody 50 μL to each well, except blank well.
7. Cover with plate cover. Incubate for 30 minutes at 37 °C.
8. Repeat the aspiration/wash process for five times as in step 5.
9. Add chromogen solution A 50 μL and chromogen solution B 50 μL to each well. Gently mix and incubate for 15 minutes at 37°C. Protect from light.
10. Add 50 μL Stop Solution to each well. The color in the wells should change from blue to yellow. If the color in the wells is green or the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
11. Read the Optical Density (O.D.) at 450 nm using a microtiter plate reader within 15 minutes.

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DATA ANALYSIS

Calculation of results

Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).
Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the x-axis against the concentration for each standard on the y-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the Rat pSmad3 concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data.
If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

Typical data

The standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.

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PRECAUTIONS

- Do not mix or substitute reagents with those from other lots or sources.
- This assay is designed to eliminate interference by other factors present in biological samples. Until all factors have been tested in the ELISA Immunoassay, the possibility of interference cannot be excluded.
- Any variation in standard diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
- When mixing or reconstituting protein solutions, always avoid foaming.
- To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- When using an automated plate washer, adding a 30 second soak period following the addition of Wash Buffer, and/or rotating the plate 180 degrees between wash steps may improve assay precision.
- Chromogen Solution is easily contaminated. If bluish prior to use, do not use.
- Stop Solution should be added to the plate in the same order as the Chromogen solution. The color developed in the wells will turn from blue to yellow upon addition of the Stop Solution. Wells that are green in color indicate that the Stop Solution has not mixed thoroughly with the Chromogen solution.
- Serum and plasma 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 Rat blood will not transmit infectious agents. Therefore, all blood derivatives should be considered potentially infectious and good laboratory practices should be followed.
- All samples should be disposed of in a manner that will inactivate viruses.
- 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.

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