Product Manual

Rat alpha-Smooth muscle actin (α-SMA) ELISA Kit

Catalog No.: abx051201

Size: 96T

Range: 0.5 ng/ml - 36 ng/ml

Sensitivity: 0.1 ng/ml

Storage: Store at 2-8 °C for 6 months.

Application: For quantitative detection of α-SMA in Rat serum, plasma, cell culture supernatant or any biological fluid.

Introduction

Alpha-actin-2 also known as actin, aortic smooth muscle or alpha smooth muscle actin (α-SMA, SMactin, alpha-SM-actin, ASMA) is a protein that in humans is encoded by the ACTA2 gene located on 10q22-q24. Actin alpha 2, the human aortic smooth muscle actin gene, is one of six different actin isoforms which have been identified. Actins are highly conserved proteins that are involved in cell motility, structure and integrity. Alpha actins are a major constituent of the contractile apparatus. Alpha-smooth muscle actin (α-SMA) is commonly used as a marker of myofibroblast formation (Nagamoto et al., 2000).

Principle of the Assay

This kit is based on sandwich enzyme-linked immuno-sorbent assay technology. Anti-α-SMA antibody is pre-coated onto 96-well plates. An HRP conjugated anti-α-SMA antibody is used as detection antibody. The standards, test samples and HRP conjugated detection antibody are added to the wells, incubated. Unbound conjugates are washed away with wash buffer. TMB substrate is used to visualize HRP enzymatic reaction. TMB is catalyzed by HRP to produce a blue color product that changes into yellow after adding acidic stop solution. The density of yellow is proportional to the α-SMA amount of sample captured in plate. The O.D. absorbance is measured spectrophoto-metrically at 450nm in a microplate reader, and then the concentration of α-SMA can be calculated.

Kit components

1. One 96-well plate pre-coated with anti-Rat α-SMA antibody
2. Rat α-SMA Standard: 0.5 ml (45 ng/ml)
3. Standard diluent buffer: 1.5 ml
4. Wash buffer (30×): 20 ml. Dilution: 1:30
5. Sample diluent buffer: 6 ml
6. HRP conjugated anti-Rat α-SMA antibody (RTU): 6ml
7. Stop solution: 6 ml
8. TMB substrate A: 6ml
9. TMB substrate B: 6ml
10. Plate sealer: 2
11. Hermetic bag: 1

Material Required But Not Provided

1. 37 °C incubator
2. Microplate reader (wavelength: 450nm)
3. Precision pipette and disposable pipette tips
4. Automated plate washer
5. ELISA shaker
6. 1.5ml tubes to prepare standard/sample dilutions.
7. Plate cover
8. Absorbent filter papers
9. 100 ml and 1 L volume graduated cylinder.

Protocol

A. Preparation of sample and reagents

1. Sample

Isolate the test samples soon after collecting and analyze immediately (within 2 hours) or aliquot and store at -20 °C or -80 °C for long term storage. Avoid multiple freeze-thaw cycles.

✧ **Cell culture supernatant:** Centrifuge at approximately 2000-3000 × g for 20 min to remove precipitant. Analyze immediately or aliquot and store at -20 °C.

✧ **Serum:** Samples should be collected into a serum separator tube. Incubate at room temperature (~ 1 hour). Centrifuge at approximately 2000-3000 × g for 20 min. Analyze immediately or aliquot and store at -20 °C.

✧ **Plasma:** Collect plasma with citrate or EDTA as the anticoagulant. Mix for 20 min approximately. Centrifuge at 2000-3000 × g for 20 min within 30 min of collection. Analyze immediately or aliquot and store at -20 °C.

✧ **Urine:** Collect in a sterile container. Centrifuge at 10,000 × g for 2 min at 4°C.

✧ **Tissues:** Rinse tissues with PBS to remove the excess of blood. Weigh, cut samples and homogenize with a tissue homogenizer in PBS (i.e. Net weight of tissue: volume of PBS=1:10, i.e. Add 9ml of PBS into 1g tissue), homogenate completely and centrifuge. Analyze immediately or aliquot and store at -20 °C.

Note:

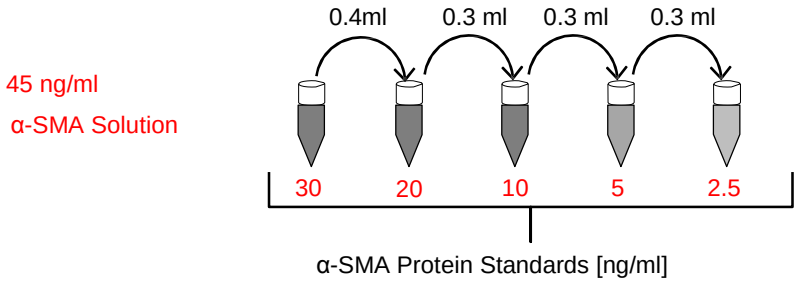
- Samples must be diluted so that the expected concentration falls within the kit's range.
- Fresh samples or recently obtained samples are recommended to prevent protein degradation and denaturalization that may lead to erroneous results.
- Coagulate blood samples completely, centrifuge, and avoid hemolysis and precipitant. Hemolysis will influence the result. Please bring samples slowly to room temperature.
- NaN_3 cannot be used as test sample preservative, since it inhibits HRP.

2. Wash buffer

Dilute the concentrated Wash buffer 30-fold (1/30) with distilled water (i.e. add 20 ml of concentrated wash buffer into 580 ml of distilled water).

3. Standard

Label 5 tubes with 30 ng/ml, 20 ng/ml, 10 ng/ml, 5 ng/ml and 2.5 ng/ml respectively. Aliquot **0.2 ml** of the Standard diluent buffer into the first two tubes labeled 30 ng/ml and 20 ng/ml respectively and **0.3 ml** of the Standard diluent buffer into each remaining tube. Add **0.4 ml** of 45 ng/ml standard solution into 1st tube and mix thoroughly. Transfer **0.4 ml** from 1st tube to 2nd tube and mix thoroughly. Transfer **0.3 ml** from 2nd tube to 3rd tube and mix thoroughly, and so on. The standard solutions are best used within 2 hours. Avoid repeated freeze-thaw cycles.



B. Assay Procedure

Equilibrate the kit components and samples to room temperature prior to use. It is recommended to plot a standard curve for each test.

1. Set standard, test sample and control (zero) wells on the pre-coated plate respectively, record their positions. It is recommended to assay standards, samples and controls in duplicate.
2. Aliquot 50 µl of the diluted standards into the standard wells.
3. Aliquot 50 µl of Standard diluent buffer into the control (zero) well. Do not add sample and HRP conjugated antibody into the control (zero) well.
4. Aliquot 50 µl of appropriately diluted sample (Rat serum, plasma or cell culture supernatants) into the test sample wells. Add the solution to the bottom of each well without touching the walls of the well. Shake the plate to mix the contents.
5. Seal the plate with a cover and incubate at 37 °C for 30 min.
6. Remove the cover and wash the plate 5 times with one of the following methods.

Manual Washing: Discard the solution without touching the side walls. Clap the plate on absorbent filter papers or other absorbent material. Fill each well completely with wash buffer and soak for 1-2 min. Aspirate the contents and clap the plate on absorbent filter papers or other absorbent material. Repeat this procedure for a total of five times.

Automated Washing: Aspirate all wells and wash the plate five times with Wash buffer (overfilling the wells with the buffer). After the final wash invert the plate and clap on absorbent filter papers or other absorbent material. It is recommended to vortex the plate mildly on ELISA shaker or allow to stand with the wash buffer for 1 min.

7. Aliquot 50 µl of HRP conjugated anti-α-SMA antibody into each well (except control well).
8. Seal the plate with a cover and incubate at 37 °C for 30 min.
9. Remove the cover and repeat the aspiration/wash process 5 times as explained in step 6.
10. Aliquot 50 µl of TMB Substrate A into each well and 50 µl of TMB Substrate B. Vortex gently the plate on ELISA shaker for 30 seconds (Or shake gently by hand for 30 seconds). Cover the plate and incubate at 37 °C for 15 min. Avoid exposure to light.
11. Aliquot 50 µl of Stop solution into each well and mix thoroughly. The color should change to yellow immediately.
12. Read the O.D. absorbance at 450 nm in a microplate reader within 15 min of adding the stop solution.

For calculation, (the relative $O.D._{450}$) = (the $O.D._{450}$ of each well) – (the $O.D._{450}$ of Zero well). The standard curve can be plotted as the relative $O.D._{450}$ of each standard solution (Y) vs. the respective concentration of the standard solution (X). The Rat α-SMA concentration of the samples can be interpolated from the standard curve.

Note: If the samples measured were diluted, multiply the dilution factor to the concentrations from interpolation to obtain the concentration before dilution.

C. Precautions

1. Before using the kit, centrifuge the tubes briefly to bring down the contents trapped in the lid.
2. Avoid foaming or bubbles when mixing or reconstituting components.
3. Wash buffer may crystallize and separate. If this happens, please warm the tube and mix gently to dissolve.
4. It is recommend to measure each standard and sample in duplicate or triplicate.
5. Do NOT let the plate completely dry at any time! This can inactivate the biological material on the plate.

6. To avoid cross contamination do not reuse pipette tips and tubes.
7. Do not use expired components or components from a different kit.
8. Store the TMB substrate B in the dark and to avoid edge effect of plate incubation for temperature differences it is recommended to equilibrate the TMB substrates for 30 min at room temperature prior to use. Aspirate the dosage needed with sterilized tips and do not dump the residual solution back into the vial.

D. Precision

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

Inter-assay Precision (Precision between assays): 3 samples with low, middle and high Rat α-SMA were tested on 3 different plates, 8 replicates in each plate.

CV (%) = SD/meanX100

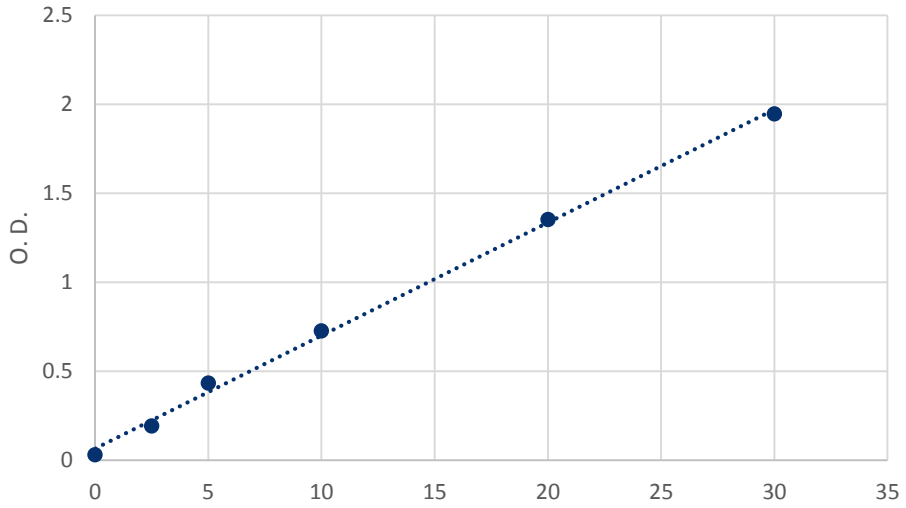
Intra-Assay: CV<10%

Inter-Assay: CV<12%

E. Typical Data & Standard Curve

Results of a typical standard run of a rat α-SMA ELISA Kit are shown below. This standard curve was generated at our lab for demonstration purpose only. Each user should obtain their own standard curve as per experiment.

ng/ml	0	2.5	5	10	20	30
OD450	0.032	0.194	0.434	0.728	1.353	1.947



This diagram is for reference only