Sheep Adrenomedullin (ADM) ELISA Kit

Catalog No.: abx055512

Size: 96T

Range: 6 pg/ml - 280 pg/ml

Sensitivity: 1 pg/ml

Storage: Store at 2-8°C for 6 months.

Application: For quantitative detection of ADM in Sheep Serum, Plasma and other biological fluids.

Introduction: Adrenomedullin (ADM or AM) is a vasodilator peptide hormone of uncertain significance in animal health and disease. AM may function as a hormone in the circulation control because it is found in blood in a considerable concentration. It was initially identified as a vasodilator, and some argued that it is the most potent endogenous vasodilatory peptide found in the body. Differences in opinion regarding the ability of AM to relax vascular tone may arise from the differences in the model system used. Other effects of AM include stimulating the growth of new blood vessels (angiogenesis) and increasing the tolerance of cells to oxidative stress and hypoxic injury. AM is seen as a positive influence in diseases such as hypertension, myocardial infarction, chronic obstructive pulmonary disease and other cardiovascular diseases, whereas it can be seen as a negative factor in potentiating the ability of cancerous cells to extend their blood supply and thereby enable further cell proliferation.

Principle of the Assay
This kit is based on sandwich enzyme-linked immuno-sorbent assay technology. Anti-ADM antibody is pre-coated onto a 96-well plate. An HRP conjugated antibody specific to ADM is used as detection antibody. The standards, test samples and HRP conjugate reagent are added to the wells and 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 intensity of the color yellow is proportional to the ADM amount bound on the plate. The O.D. absorbance is measured spectrophotometrically at 450 nm in a microplate reader, and then the concentration of ADM can be calculated.

Kit components
1. One pre-coated 96-well microplate (8 × 12 well strips)
2. Standard: 0.5 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 conjugate reagent: 6ml
7. Stop solution: 6 ml
8. TMB substrate A: 6 ml
9. TMB substrate B: 6 ml
10. Plate sealer: 2
11. Hermetic bag: 1

Material Required But Not Provided
1. 37°C incubator
2. Microplate reader (wavelength: 450 nm)
3. Precision pipette and disposable pipette tips
4. Automated plate washer
5. ELISA shaker
6. 1.5 ml tubes to prepare standard/sample dilutions
7. Plate cover
8. Absorbent filter papers
9. 100 ml and 1 L volume graduated cylinders

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.

- **Serum:** Samples should be collected into a serum separator tube. Coagulate the serum by leaving the tube undisturbed in a vertical position overnight at 4°C or at room temperature for up to 60 minutes. Centrifuge at approximately 2000-3000 × g for 20 min. Analyze the serum immediately or aliquot and store at -20°C or -80°C.
- **Plasma:** Collect plasma using citrate or EDTA as an anticoagulant. Centrifuge for 20 minutes at 2000-3000 × g within 30 minutes of collection. Assay immediately or aliquot and store at -20°C. Avoid hemolysis and high cholesterol samples.
- **Other biological fluids:** Centrifuge at approximately 1000 × g for 20 min to remove precipitant. Analyze immediately or aliquot and store at -20°C or -80°C.

Note:

» The QC data has been produced using a 1:5 dilution with the provided Sample diluent buffer. If the sample concentration is unknown, we would recommend a starting point of 1:5 dilution with the provided Sample diluent buffer.

» 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.

» Please bring sample slowly to room temperature. Sample hemolysis will influence the result. Hemolyzed specimen should not be used.

» NaN_3 cannot be used as test sample preservative, since it inhibits HRP.

» Always use non-pyrogenic, endotoxin-free tubes for blood collection.

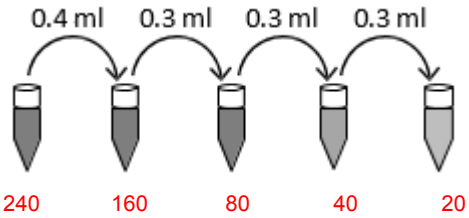
2. Wash buffer

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 240 pg/ml, 160 pg/ml, 80 pg/ml, 40 pg/ml and 20 pg/ml respectively. Aliquot 0.2 ml of the Standard diluent buffer into the first two tubes labeled 240 pg/ml and 160 pg/ml respectively and 0.3 ml of the Standard diluent buffer into each remaining tube. Add 0.4 ml of 360 pg/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.

360 pg/ml
ADM Solution



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 conjugate reagent into the control (zero) well.
4. Aliquot 50 µl of appropriately diluted sample (Sheep serum, plasma or cell culture supernatants) into the test sample wells. Add the solution at the bottom without touching the side walls of the well. Shake the plate mildly 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. Tap the plate on absorbent paper or other absorbent material. Fill each well completely with wash buffer and incubate on an ELISA shaker for 2 min. Discard the contents and tap the plate on absorbent filter papers or other absorbent material. Repeat this procedure five times.

Automated Washing: Discard the solution in 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 tap on absorbent filter papers or other absorbent material. It is recommended that the washer be set for a soaking time of 1 min.

7. Aliquot 50 µl of HRP conjugate reagent 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 the plate gently on an 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. Add 50 µl of Stop solution into each well. There should be a color change to yellow. Gently tap the plate to ensure thorough mixing.
12. Ensure that there are no fingerprints or water on the bottom of the plate, and that the fluid in the wells is free of bubbles. Measure the absorbance at 450 nm immediately.

For calculation, (relative O.D.450) = (O.D.450 of each well) – (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). Log-log curve fitting is recommended for data analysis. The Sheep ADM concentration of the samples can be interpolated from the standard curve.

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

C. Precautions

1. Before using the kit, centrifuge the tubes 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 dry out completely as this will inactivate the biological material on the plate.
6. Ensure plates are properly sealed or covered during incubation steps.
7. Complete removal of all solutions and buffers during wash steps is necessary for accurate measurement readings.
8. To avoid cross contamination do not reuse pipette tips and tubes.
9. Do not use expired components or components from a different kit.
10. 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. TMB Substrate solution is easily contaminated; sterility precautions should be taken. 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, medium and high levels of ADM were tested 20 times on one plate, respectively.

Inter-assay Precision (Precision between assays): 3 samples with low, medium and high levels of ADM were tested on 3 different plates, 8 replicates in each plate.

CV (%) = (Standard Deviation / mean) × 100

Intra-Assay: CV<10%

Inter-Assay: CV<12%