EQUINE alpha-MSH

PRODUCT PROFILE AND INSTRUCTIONS

INTENDED USE
EQUINE alpha-MSH ELISA is intended to quantitative determination of EQUINE alpha Melanocyte Stimulating Hormone (a-MSH) concentration in serum/plasma of EQUINE and related species. The test is designed as research tool in evaluation of physiological and pathological changes in the circulating a-MSH and should be employed by a trained/skilled professional.

INTRODUCTION
alpha-MSH is Peptide hormone (is also called Melanocyte Stimulating Hormone) is secreted by the intermediate lobe of pituitary gland and is under the influence of hypothalamic Corticotrophin Releasing Factor (CRF). Alpha-MSH is made up of 1-13 residues of ACTH, adrenocorticotropic hormone. There are a lot of structural similarities of A-MSH between species and for example, a-MSH promotes pigmatosis in fish and amphibians and also humans.
The EQUINE a-MSH Enzyme Immunoassay provides a rapid, sensitive and reliable results in 3 hours.

TEST PRINCIPLE
The rA-MSH Quantitative ELISA Test Kit is based on the principle of a solid phase enzyme-linked immunosorbent assay (ELISA). The assay system utilizes highly specific rabbit anti-alpha-MSH specific antibody for solid phase (microtiter wells) immobilization and a-MSH-Peptide-enzyme (horseradish peroxidase) conjugate solution. The test sample is allowed to react simultaneously with the antibodies on the plate and HRP Conjugated a-MSH molecules creating a specific competition. After 3 hours of incubation at 37ºC, the wells are washed with water to remove unbound-a-MSH HRP. A solution of TMB is added and incubated for 20 minutes, resulting in the development of a blue color. The color development is stopped with the addition of stop solution, and the color is changed to yellow and measured spectrophotometrically at 450nm. The concentration of a-MSH is inversely proportional to the color intensity of the test sample.

MATERIALS PROVIDED
1. Antibody-coated microtiter wells, 96-well plate
2. Enzyme -Conjugate reagent 12 mL
3. Reference Standards (0, 0.1,0.5, 1.0, 2.5, 10 ng/mL) 1.0ml/vial
4. TMB Color Reagent, 12 mL
5. Stop solution (2N HCl) , 6 mL
6. 20x Washing Buffer , 20 mL
7. Sample diluent, 20ml.
8. Instructions

MATERIALS REQUIRED, BUT NOT PROVIDED
1. Precision pipettes: 50uL, 100uL, 200uL, and 1.0mL
2. Disposable pipette tips
3. Distilled water
4. Glass tubes or flasks to prepare TMB Solution
5. Vortex mixer or equivalent
6. Absorbent paper
7. Graph paper
8. Microtiter plate reader

SPECIMEN COLLECTION AND PREPARATION
Serum/plasma should be prepared from a whole blood specimen obtained by acceptable medical techniques. This kit is for use with serum or plasma samples only and not for whole blood. The EQUINE test samples (plasma or serum) should be collected fresh and repeated freeze and thaw should be avoided, If the test samples are not analyzed immediately, should be stored at -20C in small aliquotes and take one aliquot at a time for analysis.

STORAGE OF TEST KIT AND INSTRUMENTATION
Unopened test kits should be stored at 4-8^{O}C upon receipt and the microtiter plate should be kept in a sealed bag with desiccants to minimize exposure to damp air. Opened test kits will remain stable until the expiration date shown, provided it is stored as prescribed above. A microtiter plate reader with a bandwidth of 10nm or less, with a bandwidth of 10nm or less and an optical density range of 0-2 OD or greater at a 450nm wavelength is acceptable for use in absorbency measurement.

REAGENT PREPARATION
1. All reagents should be brought to room temperature (18-25^{O}C) before use.
2. Ready to use reference standards is provided with the kit. The standards are stable at 2-8^{O}C for 2 weeks or kept frozen at -20C for long term use.

ASSAY PROCEDURE

One must follow accurately these steps to ensure correct results. Use clean pipettes and disposable tips

1. Secure desired number of coated wells in the holder.
2. Dispense 150 ul of standards, specimens, and controls into appropriate wells.
3. Dispense 50ul of Enzyme Conjugate Reagent into each well. Mix for 30 seconds. It is very important to have completed mixing at this step.
4. Incubate at 37C for 3 hours.
5. Remove the incubation mixture by dumping plate contents into a waste container.
6. Rinse and dump the microtiter wells five (5) times (300ul) with dilute wash buffer.
7. Dispense 100 ul of TMB solution into each well. Gently mix for 10 seconds.
8. Incubate at room temperature for 20 minutes in the dark.
9. Stop reaction by adding 50 ul of stop solution (2N HCl) to each well.
10. Gently mix for 30 seconds. It is important to observe a color change from blue to yellow.
11. Read optical density at 450nm with a microtiter well reader.

Important note: The wash steps are very critical and insufficient washing will result in poor precision and falsely elevated absorbency readings.

CALCULATION OF RESULTS

Calculate the mean absorbency value (A450) for each set of reference standards, specimens, controls and test samples. Construct a standard curve by plotting the mean absorbency obtained from each reference standard against its concentration in ng/ml on graph, with absorbency values on the vertical or Y axis, and concentrations on the horizontal or X axis. Use the mean absorbency values for each specimen to determine the corresponding concentration of rA-MSH ng/mL from the standard curve.

EXPECTED VALUES AND SENSITIVITY

It is recommended to establish your local laboratory conditions for normal range in your laboratory animals.
Minimum detectable levels in this assay will be 0.1ng/ml

LIMITATIONS OF THE PROCEDURE

Reliable and reproducible results will be obtained when the assay procedures are carried out with understanding of the package insert instructions and adherence to good laboratory practice.
The wash step is extremely important and should follow for clean background and good reproducible results. Incubation conditions should be carefully monitored or establishing conditions at 37C should make adjustments for consistent and reproducible results.
Note: The components of this kit should not be mixed are used with other manufacturer kits.

REFERENCES

1. Fisher DA 1977 Evaluation of anterior pituitary function In Radioimmunoassay Manual Eds. Nicholas AL and Nelson JCP 3498 Nichols Institute.
2. Reichlin S et al. 1976 Hypothalamic hormones Ann Rev Med 27p359

35325 Fircrest Street, Newark, CA 94560-1003 * Phone (800) 745-0843 * (510) 745-0844 * Fax (510) 745-0977

A TYPICAL ALPHA EQUINE ALPHA MSH ELISA STANDARD CURVE