CYO («Coat Your Own») ELISA Kit
Horse proAKAP4
Reference: 4VDX-17C3

The Horse pro A-kinase anchor protein 4 / proAKAP4 «Coat Your Own» ELISA Kit (CYO) contains the key components required for quantification of horse proAKAP4 in fresh ejaculates or in frozen / diluted semen. The components provided in this kit are sufficient to run 40-samples and 8 standards in duplicate.

REAGENTS PROVIDED: 4 VIALS AND ONE 96-WELLS ELISA

1 Vial of Capture Antibody: The vial contains 100 µL of capture antibody (250 µg/mL).
1 Vial of Lyophilized Standard: One vial at 10 µg of Standard.
1 Vial of Detection Antibody: The vial contains 60 µL of horseradish-conjugated antibody (100 µg/mL) for detection of Horse proAKAP4.
1 Vial of Horse Semen Lysis Buffer: The vial contains 10 mL of Horse Semen Lysis Buffer.
1 Microplate: An ELISA plate of 96-wells (12x8 strips).

SOLUTIONS REQUIRED - NOT INCLUDED

Buffer A: Carbonate/Bicarbonate Buffer 0.1 M, pH 9.6.
Buffer B: 10 mM ethanolamine in Buffer A.
Buffer C: PBS containing 0.05% Tween-20.
Buffer D: PBS containing 0.04% casein.
Substrate solution: TMB Substrate Solution.
Stop solution: 2N H_2SO_4 or 4N HCl.

MATERIAL REQUIRED - NOT INCLUDED

Sonicator: minimum of 20kHz, 100W, probe of 2 to 3 mm.
Tubes: Polypropylene tubes for dilution.
Shaker: Horizontal orbital microplate shaker.
Microplate reader capable of measuring absorbance at 450 nm and with the correction wavelength set to 630 nm.
Multichannel Pipette.
Pipette of 20 µL, 200 µL and 1000 µL and disposable tips.

STORAGE INFORMATION

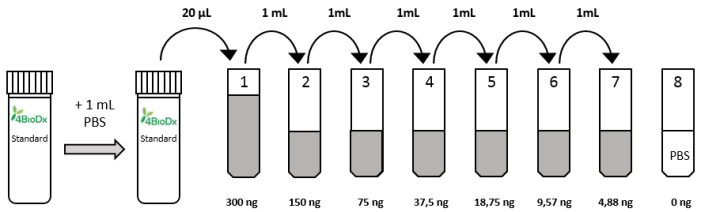
Store reagents when unopened at 4°C.

DAY-1: COATING OF ELISA PLATE

1. Dilute the Capture Antibody to a working concentration of 1.5 µg/mL in Buffer A.
2. Add 100 µL of Capture Antibody per well.
3. Seal/cover the plate with an adhesive sealer.
4. Incubate overnight at 4°C.

DAY-2: STANDARD PREPARATION FOR ASSAY

1. Rapidly centrifuge the Lyophilized Standard.
2. Carefully open the vial and reconstitute the Standard by adding 1 mL of Buffer D to obtain a solution at 10 µg / mL.
3. Prepare the highest concentration of Standard (50 ng/mL) by pipetting 20 µL of the reconstituted Standard solution into 1,98 mL of Buffer D.
4. Add 1 mL of Buffer D into 6 tubes (always use polypropylene tubes).
5. Perform a serial dilution by pipetting 1 mL of the first tube (50 ng/mL) and adding it to the second tube already containing 1 mL of Buffer D (2-fold dilution), mix thoroughly.
6. Then take 1 mL from the second tube and add it to third tube and mix. Reproduce until the seventh tube. Buffer D serves as the zero standard (0 ng/mL).



DAY-2: SEMEN SAMPLE PREPARATION AND DILUTION FOR ELISA

ProAKAP4 biomarker should be first extract from spermatozoa by using a specific lysis buffer.

Fresh Ejaculate:

1. In a 0.5 mL conic tube add 20 µL of Horse Semen to 180 µL of Horse Semen Lysis Buffer to reach a final volume of 200 µL.
2. Vortex.
3. Sonicate 1 min at 60% amplitude (sonicator characteristics, 20 kHz, 100W, probe of 2 to 6 mm).
4. Add 200 µL of Buffer D.
5. Vortex.
6. Keep on ice until use.

Frozen or refrigerate semen in extenders:

1. In a 1.5 mL conic tube add 40 µL of frozen or refrigerate Semen to 160 µL of Horse Semen Lysis Buffer to reach a final volume of 200 µL.
2. Vortex.
3. Sonicate 1 min at 60% amplitude (sonicator characteristics, 20 kHz, 100W, probe of 2 to 6 mm).
4. Add 200 µL of Buffer D.
5. Vortex.
6. Keep on ice until use.

DAY-2: SANDWICH ELISA PROTOCOL

1. Remove the Capture Antibody by inverting the plate.
2. Wash each well three times with 300 µL of Buffer A.
3. After the last wash, remove any remaining Buffer A by inverting the plate against a clean paper towel.
4. Add 300 µL of Buffer B to each well and wait for 1 h at RT (20 to 25°C).
5. Wash three times each well with 300 µL of Buffer C.
6. After the last wash and removal of the Buffer C, add 100 µL of each Standard dilution and Sample in duplicate.
7. Cover the plate with a plate sealer and incubate for 2h at RT with gentle agitation (300 rpm).
8. Wash three times each well with 300 µL of Buffer C.
7. Dilute the Detection Antibody to a working concentration of 0.5 µg/mL in Buffer D.
8. Add 100 µL of Detection Antibody per well.
9. Cover the plate with a plate sealer and incubate for 1 h at RT with gentle agitation (300 rpm).
9. Wash three times each well with 300 µL of Buffer C.
10. Add 100 µL of Substrate Solution to each well. Keep away from light.
11. Incubate for 10 minutes at RT until the blue coloration appears.
12. Add 50 µL of Stop Solution to each well.
13. Determine the optical density using a microplate reader set to 450 nm and with wavelength correction set to 630 nm.
14. Create a standard curve by reducing the data using a computer software generating a four-parameter logistic curve fit. If Samples have been diluted, the concentration read from the standard curve must be adjusted by multiplying the values by the dilution factor.

CALCULATION OF RESULTS

Average the duplicate optical density measures for each Standard or Sample and subtract optical density of zero point of the peptide standard dilution to the optical density of each optical density of Standard or Sample.

REFERENCES

Sergeant et al. (2016) Animal Reproduction Science. Vol.169:125-126

Novak et al. (2010) Theriogenology. Vol.74:956-967

Leeb et al. (2005) Animal Reproduction Science. Vol.89:21-29

Turner et al. (2005) American Journal of Vet. Research. Vol.66:1055-1056

FOR RESEARCH USE ONLY
NOT FOR USE IN DIAGNOSTIC PROCEDURES

Distribuito in ITALIA da
Li StarFish S.r.l.
Via Cavour, 35
20063 Cernusco S/N (MI)
telefono 02-92150794
fax 02-92157285
info@listarfish.it
www.listarfish.it