Horse proAKAP4 ELISA kit

Reference: 4VDX-18K3

PRINCIPLE AND GENERAL DESCRIPTION

The horse proAKAP4 ELISA kit (4VDX-18K3) is a quantitative sandwich ELISA assay to detect and quantify the biomarker proAKAP4 in sperm samples. This kit is composed of a 96-well plate coated with a monoclonal antibody specific for proAKAP4. Samples like fresh, frozen or dilute horse semen are lysed with the Horse Spermatozoa Lysis Buffer and then pipetted into the wells. Then proAKAP4 will bind to the antibody-coated plate and be detected using a Detection Antibody covalently coupled to horseradish peroxidase. A Substrate Solution is added to each well and color level appears proportionally to the amount of proAKAP4. The color reaction is stopped by the Stop Solution and the color intensity is measured by spectrophotometry at 450 nm. A Lyophilized Standard is provided to perform a reference curve enabling to determine the concentration of the proAKAP4 present in each individual sperm sample.

REAGENTS

R1 - Microplate: An ELISA plate of 96-wells (12x8 strips)
R2 - 1 vial of 10x Washing Buffer Solution
R3 - 1 vial of 1x Dilution Buffer
R4 - 1 Vial of Lyophilized Standard
R5 - 1 Vial of 1x Lysis Buffer of Horse Spermatozoa
R6 - 1 Vial of Detection Antibody
R7 - 1 Vial of Substrate Solution
R8 - 1 Vial 1x Stop Solution

STORAGE INFORMATION

At reception all components of the ELISA kit should be stored at 4°C.

MATERIAL REQUIRED

Sonicator*: minimum of 20kHz, 100W, sonication probe of 2 to 3 mm.
Tubes: Polypropylene tubes for dilution.
Shaker: Horizontal orbital microplate shaker.
Microplate reader 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.
Pipette tips.
Deionized water.

* Alternatively, you may use glass or ceramic beads – dedicated protocol on request

BUFFER AND STANDARD PREPARATION FOR ASSAY

1. Prepare the 1x Washing Buffer by a 10-fold dilution of the 10x concentrated R2 Washing Buffer Solution: 30 mL to 270 mL.

2. Rapidly centrifuge the R4 Lyophilized Standard.

2. Carefully open the vial and reconstitute the R4 Lyophilized Standard by adding 1 mL of R3 Dilution Buffer and obtain a solution at 10 µg / mL.

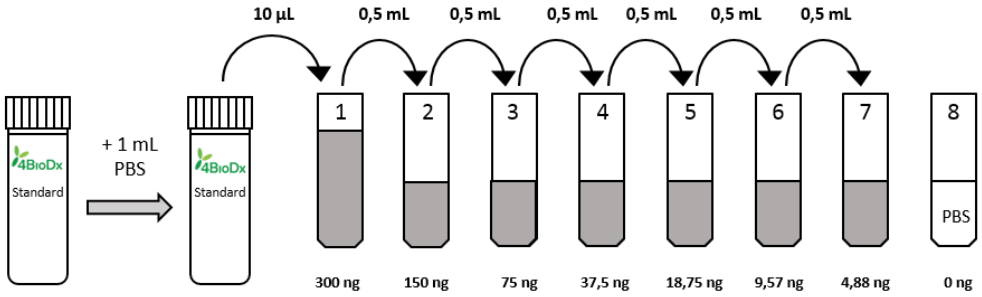
3. Prepare the highest concentration of Standard (100 ng/mL) by pipetting 10 µL of the reconstituted Standard Solution into 990 µL of R3 Dilution Buffer.

4. Add 0.5 mL of R3 Dilution Buffer into 6 tubes (always use polypropylene tubes).

5. Perform a serial dilution by pipetting 500 µL of the first tube (100 ng/mL) and adding it to the second tube already containing 500 µL of Buffer D (2-fold dilution), mix thoroughly.

6. Then take 500 µL 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).

The equivalent of proAKAP4 quantity measured by well is 300, 150, 75, 37.5, 18.75, 9.57, 4.88 and 0 ng of proAKAP4 in the serial dilution. These values are used to perform the standard curve.



SEMEN SAMPLE PREPARATION AND DILUTION FOR ELISA

AKAP4 biomarker should be first extract from spermatozoa by using a specific Lysis Buffer.

Fresh Ejaculate:

1. In a 0.5 mL conic tube add 260 µL of Horse Lysis Buffer

2. Add 40 µL of semen to the R5 Lysis Buffer to reach a volume of 300 µL

3. Vortex.

4. Add 300 µL of R3 Dilution Buffer

5. Vortex.

6. Sonicate 1 min at 60% amplitude (sonicator characteristics, 20 kHz, 100W, probe of 2 to 6 mm).

7. Keep on ice until use.

Frozen semen:

Unfroze the semen sample at 4°C or on ice, resuspend spermatozoa by mixing the semen and proceed as for fresh ejaculate.

Frozen or refrigerate semen in extenders:

1. In a 0.5 mL conic tube add 240 µL of R5 Lysis Buffer

2. Add 60 µL of Semen to the R5 Lysis Buffer to reach a volume of 300 µL

3. Vortex.

4. Add 300 µL of R3 Dilution Buffer.

5. Vortex.

6. Sonicate 1 min at 60% amplitude (sonicator characteristics, 20 kHz, 100W, probe of 2 to 6 mm).

7. Keep on ice until use.

ELISA PROTOCOL

1. Add 100 µL of each Standard Dilution and Sample in duplicate.

3. Cover the plate with a plate sealer and incubate for 2h at RT with gentle agitation (250 rpm).

4. Wash three times each well with 300 µL of R2 Washing Buffer 1x solution.

5. Dilute the 60 µL of Detection Antibody in 12 mL of R3 Dilution Buffer.

6. Add 100 µL of Detection Antibody per well.

7. Cover the plate with a plate sealer and incubate for 1 h at RT with gentle agitation (250 rpm).

8. Wash three times each well with 300 µL of R2 Washing Buffer 1x Solution.

9. Add 100 µL of R7 Substrate Solution to each well. Keep away from light.

10. Protect from light and incubate under gentle agitation (300 rpm) for 10 minutes at RT.

11. Add 50 µL of R8 Stop Solution to each well.

12. Determine the optical density using a microplate reader set to 450 nm and with wavelength correction set to 630 nm.

13. 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. Then substract optical density of zero point of the peptide standard dilution to the optical density of each optical density of Standard or Sample.

CAUTIOUS

- Always wear gloves and protection glasses and follow the good laboratory practice.
- Use the whole chemicals before the expiration limit.
- Do not pipette with mouth.
- The Substrate Solution can be irritating for the skin.
- The Stop Solution can be harmful in case of ingestion et could lead when in contact with the skin to irritation. Please avoid the contact with skin.
- Do not expose the substrate solution to light nor to oxidative substances.
- Observe all federal, state, and local regulations for disposal.
- The user should calculate the possible amount of the samples used in the whole test. Please make sure that sufficient samples are available.
- The kit cannot assay the samples which contain sodium azide (NaN3), because NaN3 will inhibit the activity of horseradish peroxidase (HRP).
- Please return the unused wells to the foil pouch containing the desiccant pack and reseal and reseal with tape. The remaining reagents still need to be stored at 2°C-8°C.
- Spin down for one or two seconds to concentrate the Standard into the bottom of the vial.

- Protect all reagents from strong light during storage and incubation.
- All bottle caps of reagents should be covered tightly to prevent the evaporation and contamination of microorganism.
- Any variation in ambient temperature, equipment, pipetting, washing, incubation time can cause variation in result. Each user should obtain his own standard curve.

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

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NOT FOR USE IN DIAGNOSTIC PROCEDURES

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