

Ram 4MID kit : (Ram proAKAP4)



Reference: 4VDX-18K7

PRINCIPLE AND GENERAL DESCRIPTION

The 4MID kit (4BDX-18K7) 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 ram semen are lysed with the Ram Spermatozoa Lysis Buffer and then pipetted into the wells. Then, proAKAP4 will be recognized by the capture antibody of the antibody-coated plate and will be detected using a Detection Antibody covalently coupled to horseradish peroxidase. A Substrate Solution is added to each well and color levels appear 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 Ram Spermatozoa Lysis Buffer**
- R6 - 1 Vial of Detection Antibody**
- R7 - 1 Vial of Substrate Solution**
- R8 - 1 Vial 1x Stop Solution**



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STORAGE INFORMATION

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

MATERIAL REQUIRED



- Tubes:** Polypropylene tubes for dilution.
- Shaker:** Horizontal orbital microplate shaker.
- Microplate reader** measuring absorbance at 450 nm.
- Vortex mixer** for preparation samples.
- Sonicator** (22kHz, 3 mm probe) (optional).
- Multichannel Pipette.**
- Pipette of 20 µL, 200 µL and 1000 µL.**
- Pipette tips.**
- Deionized water.**

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 into 270 mL of deionized water.
2. Rapidly centrifuge the R4 Lyophilized Standard.
3. 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.

4. Prepare the highest concentration of Standard by pipetting 10 μL of the reconstituted Standard Solution into 1990 μL of R3 Dilution Buffer.
5. Add 0.5 mL of R3 Dilution Buffer into 6 tubes (always use polypropylene tubes).
6. Perform a serial dilution by pipetting 500 μL of the first tube and adding it to the second tube already containing 500 μL of Buffer D (2-fold dilution), mix thoroughly.
7. 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 150, 75, 37.5, 18.75, 9.37, 4.68, 2.34 and 0 ng of proAKAP4 in the serial dilution. These values are used to perform the standard curve.

		10 μL	0.5 mL	0.5 mL	0.5 mL	0.5 mL	0.5 mL	0.5 mL	
		1	2	3	4	5	6	7	8
	+ 1 mL PBS								
									PBS
		150 ng	75 ng	37.5 ng	18.75 ng	9.37 ng	4.68 ng	2.34 ng	0 ng

SEMEN SAMPLE PREPARATION AND DILUTION FOR ELISA

ProAKAP4 biomarker should be first extract from spermatozoa by using a specific Ram Spermatozoa Lysis Buffer.

Fresh Ejaculate:

1. In a 0.5 mL conic tube add 270 μL of Ram Spermatozoa Lysis Buffer.
2. Add 30 μL of semen to the R5 Ram Spermatozoa Lysis Buffer to reach a volume of 300 μL .
3. Vortex 1 min at maximum speed or sonicate at 50 % of amplitude with a 3 mm probe at 22 kHz during 30 second.
4. Add 300 μL of R3 Dilution Buffer.
5. Vortex 1 min at maximum speed.
6. Keep at ambient temperature (17°C – 25°C). Store at -20°C if you store for longer than 1 hour.

Frozen semen:

Unfroze the semen sample and proceed as for fresh ejaculate.

Frozen or refrigerate semen in extenders:

1. In a 0.5 mL conic tube add 250 μL of R5 Ram Spermatozoa Lysis Buffer.
2. Add 50 μL of Semen to the R5 Ram Spermatozoa Lysis Buffer to reach a volume of 300 μL .
3. Vortex 1 min at maximum speed or sonicate at 50 % of amplitude with a 3 mm probe at 22 kHz during 30 second.
4. Add 300 μL of R3 Dilution Buffer.
5. Vortex 1 min at maximum speed.
6. Keep at ambient temperature (17°C – 25°C). Store at -20°C if you store for longer than 1 hour.

ELISA PROTOCOL

1. Add 100 μ L of each Standard Dilution and Sample in duplicate.
2. Cover the plate with a plate sealer and incubate for 1h at RT with gentle agitation (250 rpm).
3. Wash three times each well with 300 μ L of R2 Washing Buffer 1x solution.
4. Dilute the 60 μ L of Detection Antibody in 12 mL of R3 Dilution Buffer.
5. Add 100 μ L of Detection Antibody per well.
6. Cover the plate with a plate sealer and incubate for 30 min at RT with gentle agitation (250 rpm).
7. Wash three times each well with 300 μ L of R2 Washing Buffer 1x Solution.
8. Add 100 μ L of R7 Substrate Solution to each well. Keep away from light.
9. Protect from light and incubate under gentle agitation (300 rpm) for 10 minutes at RT.
10. Add 50 μ L of R8 Stop Solution to each well.
11. Determine the optical density using a microplate reader set to 450 nm and with wavelength correction set to 630 nm.
12. 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. The results are expressed as the concentration of proAKAP4 in ng / μ L of semen.

CALCULATION OF RESULTS

Average the duplicate optical density measures for each Standard or Sample. Then subtract 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 (NaN_3), because NaN_3 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

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- **Sergeant et al. (2016)** Animal Reproduction Science. Vol.169:125-126
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- **Miki et al. (2002)** Developmental Biology. Vol.248:331-342

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NOT FOR USE IN DIAGNOSTIC PROCEDURES



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