



ELISA PRODUCT INFORMATION & MANUAL

Sheep IL-5 ELISA Development Kit

NBP3-18277

Enzyme-linked Immunosorbent Assay for quantitative
detection. For research use only.

Not for diagnostic or therapeutic procedures.

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Novus kits are guaranteed for 6 months from date of receipt

Sheep IL-5 ELISA Development Kit

ELISA Flex kit for quantitative determination of native sheep IL-5 in solution, e.g. cell supernatant and serum/plasma.

The kit includes		NBP3-18277 for 6 plates	
Capture mAb:	MT52B34 (0.5 mg/ml)	300 µl	
Detection mAb:	MT11B37, biotinylated (0.5 mg/ml)	150 µl	
Streptavidin-HRP		80 µl	
Recombinant bovine IL-5 ELISA standard		1 vial	
Standard reconstitution buffer A5		1 ml	

To ensure total recovery of the stated quantity, vials have been overfilled.

Shipping and storage

Shipped at ambient temperature. All reagents should be stored at 4-8 °C upon receipt, except the standard which should be stored at -20 °C. Antibodies are supplied in sterile-filtered PBS with sodium azide (0.02%). Streptavidin-HRP is supplied in PBS with 0.002% Kathon CG. The expiry date indicates how long unopened products, stored according to instructions, are recommended for use.

General and Preparations

Specificity

The kit contains a matched pair of monoclonal antibodies (mAbs) specific for native and recombinant bovine IL-5. The mAbs cross-react with IL-5 from sheep. The ELISA standard included is recombinant bovine IL-5.

Standard range

4-1000 pg/ml

Calibration

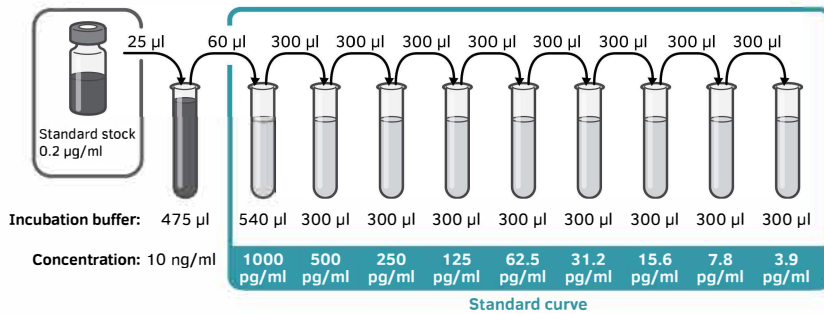
No international standard exists for calibration.

Reconstitution of ELISA standard

Reconstitute the ELISA standard to a stock solution of 0.2 µg/ml by adding 1 ml of the standard reconstitution buffer. Allow the standard to dissolve for 5 minutes and mix thoroughly. The standard should be kept in aliquots at -20 °C. Avoid repeated freeze-thaw cycles.

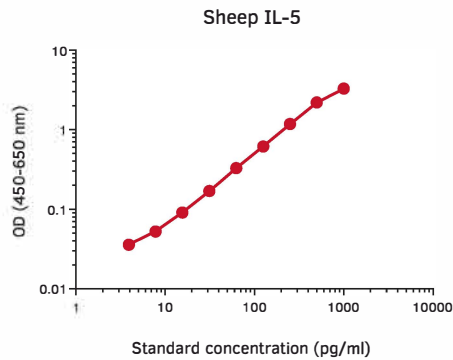
Preparation of standard curve

Prepare within 30 minutes of use. Volumes are sufficient for duplicates.



Protocol

- Day 1**
1. Add 100 μl /well of capture mAb MT52B34 diluted to 2 $\mu\text{g}/\text{ml}$ in PBS, pH 7.4. Use high protein binding ELISA plates. Incubate overnight at 4-8 $^{\circ}\text{C}$.
- Day 2**
2. Empty the plate and add 200 μl /well of PBS with 0.05% Tween 20 and 0.1% BSA (incubation buffer) to block the plate. Incubate for 1 hour at room temperature.
 3. Wash the plate 5 times with PBS containing 0.05% Tween 20 (300 μl /well).
 4. Add 100 μl /well of samples or standards diluted in incubation buffer. Include assay background control, i.e. wells without standard. Incubate for 2 hours at room temperature.
 5. Wash as above.
 6. Add 100 μl /well of detection mAb MT11B37-biotin diluted to 1 $\mu\text{g}/\text{ml}$ in incubation buffer. Incubate for 1 hour at room temperature.
 7. Wash as above.
 8. Add 100 μl /well of Streptavidin-HRP diluted 1:1000 in incubation buffer. Incubate for 1 hour at room temperature.
 9. Wash as above.
 10. Add 100 μl /well of TMB substrate and incubate for 15 minutes.
 11. Add 100 μl /well of 0.2 M H_2SO_4 to stop the reaction.
 12. Measure the optical density in an ELISA reader at 450 nm within 15 min. Preferably use a reader capable of subtracting a reference wavelength of between 570 and 650 nm. Representative standard curve shown below.



Quality management system complies with the standards
ISO 9001:2015 & ISO 13485:2016.



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