Datasheet & Protocol



ELISA Flex: Goat IL-5 (HRP)

3193-1H-6 | 3193-1H-20

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

The kit includes		3193-1H-6 for 6 plates	3193-1H-20 for 20 plates
Capture mAb:	MT52B34 (0.5 mg/ml)	300 μΙ	1000 μΙ
Detection mAb:	MT11B37, biotin (0.5 mg/ml)	150 μΙ	500 μΙ
Streptavidin-HRP		80 μΙ	250 μΙ
Recombinant bovine IL-5 ELISA standard		1 vial	1 vial
Standard reconstitution buffer A5		1 ml	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 goat. The ELISA standard included is recombinant bovine IL-5.

Standard range

4-1000 pg/ml

Calibration

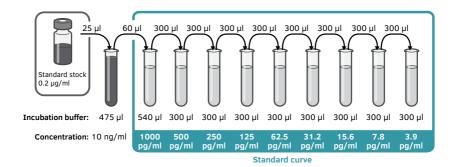
No international standard exists for calibration.

Reconstitution of ELISA standard

Reconstitute the ELISA standard to a stock solution of 0.2 μ g/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.



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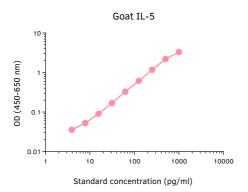
Protocol

Day 1

1. Add 100 μ l/well of capture mAb MT52B34 diluted to 2 μ g/ml in PBS, pH 7.4. Use high protein binding ELISA plates. Incubate overnight at 4-8 °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 μ l/well of detection mAb MT11B37-biotin diluted to 1 μ g/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. Please note that sodium azide used in buffers will inhibit HRP activity.
- **9.** Wash as above.
- **10.** Add 100 μl/well of TMB substrate (product code: 3652-F10) and incubate at room temperature, protected from direct light for 15 minutes.
- **11.** Add 100 μ l/well of 0.2 M H₂SO₄ 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.



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Mabtech AB (Head Office) Sweden

Tel: +46 8 716 27 00 mabtech@mabtech.com Mabtech, Inc.

USA Tel: +1 513 871-4500 mabtech.usa@mabtech.com