# ELISA PRODUCT INFORMATION & MANUAL

# CXCL8/IL-8 ELISA Development Kit

## *NBP3-11747*

Enzyme-linked Immunosorbent Assay for quantitative detection. For research use only.
Not for diagnostic or therapeutic procedures.

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# CXCL8/IL-8 ELISA DEVELOPMENT KIT

ELISA Development Kit for quantitative determination of native or recombinant bovine CXCL8/IL-8 in solution, e.g. cell supernatant and serum/plasma samples.

| The kit includes | | NBP3-11747 for 6 plates |
|---|---|---|
| Capture mAb: | MT8H6 (0.5 mg/ml) | 300 µl |
| Detection mAb: | 26E5, biotinylated (0.5 mg/ml) | 50 µl |
| Streptavidin-HRP | | 80 µl |
| Recombinant bovine IL-8 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 human CXCL8/IL-8 The mAbs cross-react with CXCL8/IL-8 from cow, monkey and dog.

## Standard range

8-800 pg/ml

## Calibration

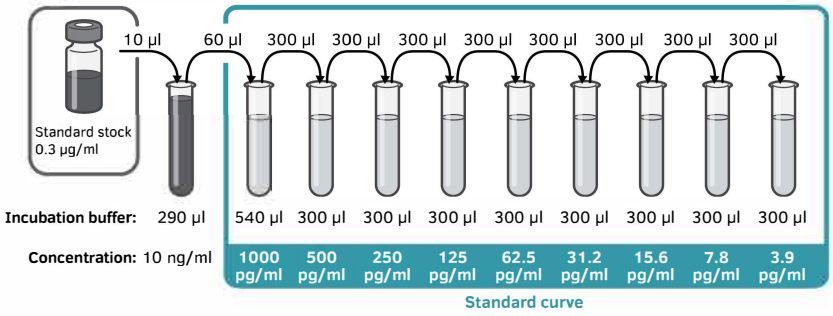
No international standard exists for calibration.

## Reconstitution of ELISA standard

Reconstitute the ELISA standard to a stock solution of 0.3 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 MT8H6 diluted to 2 µg/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 µl/well of detection mAb 26E5-biotin diluted to 0.1 µg/ml in incubation buffer. Incubate for 1 hour at room temperature.

**7.** Wash as above.

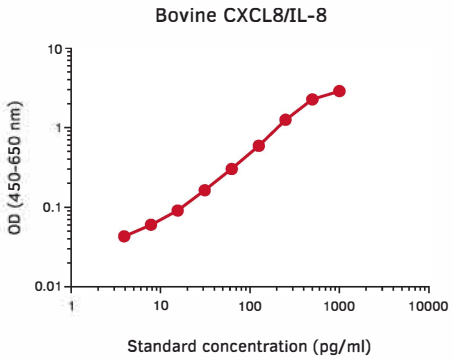
**8.** Add 100 µl/well of Streptavidin-HRP diluted 1:1000 in incubation buffer. Incubate for 1 hour at room temperature. Please note that sodium azide used in buffers will inhibit HRP activity.

**9.** Wash as above.

**10.** Add 100 µl/well of TMB substrate 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.



Updated on 2022/02/23

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

**The products are for research use only.**