# Datasheet & Protocol



# ELISA Flex: Monkey IL-8 (HRP)

3560M-1H-6 | 3560M-1H-20

ELISA Flex kit for quantitative determination of native monkey IL-8 (CXCL8) in solution, e.g. cell supernatant.

The kit includes	<b>3560M-1H-6</b> for 6 plates	<b>3560M-1H-20</b> for 20 plates
Capture mAb: MT8H6 (0.5 mg/ml)	300 μl	1000 μΙ
Detection mAb: 26E5, biotin (0.5 mg/ml)	50 μΙ	150 μΙ
Streptavidin-HRP	80 μΙ	250 μΙ
Recombinant human IL-8 (CXCL8) 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 human IL-8 (CXCL8). The mAbs cross-react with IL-8 from non-human primates. Please visit www.mabtech.com for reactivity on NHP species. The mAbs also cross-react with IL-8 from cow, thus the use of bovine serum in cell-cultures is not recommended.

## Standard range

8-800 pg/ml

#### Calibration

No international standard exists for calibration.

# Analysis of serum and plasma samples

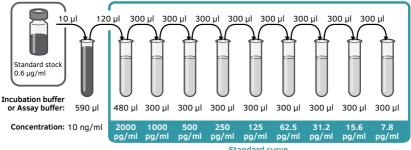
Analysis of serum/plasma requires the use of Assay buffer (product code: 3652-J2). The Assay buffer blocks heterophilic antibodies, commonly found in serum/plasma, from cross-linking the assay antibodies, thereby preventing false positive read-outs. The Assay buffer should be used for dilution of standard, samples, and detection antibody.

#### Reconstitution of ELISA standard

Reconstitute the ELISA standard to a stock solution of 0.6 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 aliguots at -20 °C. Avoid repeated freeze-thaw cycles.

#### Preparation of standard curve

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



Standard curve

2 www.mabtech.com

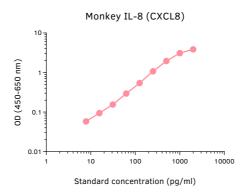
# **Protocol**

## 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  $\mu$ l/well).
- **4.** Add 100 µl/well of samples or standards diluted in incubation buffer or Assay 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 or Assay 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  $\mu$ l/well of 0.2 M H<sub>2</sub>SO<sub>4</sub> 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.



3 www.mabtech.com



Developed and manufactured by MABTECH AB, Sweden, whose quality management system complies with the standards ISO 9001:2015 & ISO 13485:2016.





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

MABTECH shall not be liable for the use or handling of the product or for consequential, special, indirect or incidental damages there from.

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