## Datasheet \& Protocol

## ELISA Flex: <br> Human IgG (ALP)

## 3850-1AD-6 |

ELISA Flex kit for quantitative determination of native human IgG in solution, e.g. serum/plasma samples or cell supernatants.

|  | 3850-1AD-6 <br> for 6 plates |  |
| :--- | :--- | :---: | :--- |
| The kit includes | $300 \mu \mathrm{l}$ |  |
| Capture mAb: MT145 (0.5 mg/ml) | $80 \mu \mathrm{l}$ |  |
| Detection mAb: MT78, ALP | 1 vial |  |
| Human IgG ELISA standard | 1 ml |  |
| Standard reconstitution buffer A5 |  |  |

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 $4-8^{\circ} \mathrm{C}$ upon receipt, except the standard which should be stored at $-20^{\circ} \mathrm{C}$. Antibodies are supplied in sterile-filtered PBS with sodium azide (0.02\%). The detection antibody is supplied in 0.1 M Tris-buffer with $1 \%$ BSA and $0.002 \%$ 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 the Fc part of human IgG. The mAbs cross-react with IgG from non-human primates (NHP). Please visit www.mabtech.com for reactivity on NHP species.

## Standard range

$0.2-100 \mathrm{ng} / \mathrm{ml}$

## Calibration

The ELISA standard has been calibrated against an international standard from the National Institute of Biological Standards and Control (NIBSC), Potters Bar, Hertfordshire EN6 3QG, UK. One $\mu \mathrm{g}$ of supplied standard equals 11 mU NIBSC-standard. Please note that the calibration is batch specific.

## Reconstitution of ELISA standard

Reconstitute the ELISA standard to a stock solution of $50 \mu \mathrm{~g} / \mathrm{ml}$ by adding 0.5 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 $-20^{\circ} \mathrm{C}$. 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 $100 \mu \mathrm{l} /$ well of capture mAb MT145 diluted to $2 \mu \mathrm{~g} / \mathrm{ml}$ in PBS, pH 7.4 . Use high protein binding ELISA plates. Incubate overnight at $4-8^{\circ} \mathrm{C}$.

## Day 2

2. Empty the plate and add $200 \mu /$ 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 $20(300 \mu / / w e l l)$.
4. Add $100 \mu \mathrm{l} /$ 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 $100 \mu \mathrm{l} /$ well of detection mAb MT78-ALP diluted $1: 1000$ in incubation buffer. Incubate for 1 hour at room temperature.
7. Wash as above.
8. Add $100 \mu / /$ well of pNPP substrate (product code: 3652 -P10) and incubate the plate for approximately 60 minutes.
9. Measure the optical density in an ELISA reader at 405 nm . Preferably use a reader capable of subtracting a reference wavelength of between 570 and 650 nm . Representative standard curve shown below.

Human IgG


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