Affinity Immuno

# GCSF-PEG ADA ELISA
# Catalog EL-141-73196-PEG

For the determination antibodies to GCSF-PEG in serum and plasma.

## Introduction

The AffinityImmuno GCSF-PEG ADA ELISA kit contains the components required for measuring antibodies to GCSF-PEG in biological matrices such as human serum, plasma or cell culture.

## Principle of the assay

This assay employs the indirect enzyme immunoassay technique. GCSF-PEG is coated onto a 96 well microplate. Calibrator and test samples are prepared by dilution into assay buffer and are pipetted into the appropriate wells. Antibodies to GCSF-PEG present in biological matrices are bound to the immobilized GCSF-PEG. After washing away any unbound substances, antibody-enzyme reagent and a substrate solution is added to the wells for color development is proportional to the amount of antibodies to GCSF-PEG present in the calibrator and test samples. The color development is stopped and the intensity of the color is measured.

## Materials and storage

Store kit components at -20°C unless specified otherwise. DO NOT USE past kit expiration date. Some vials contain a small amount of reagents. Spin tubes on pulse setting prior to opening.

| Each kit includes: | Count |
|---|---|
| Coated microtiter plate, 96 wells (12x8 strips) | 1 |
| Calibrator diluent | 1.8 ml |
| Calibrator (1.15mg/mL) | 12 µl |
| 10X wash buffer | 50 ml |
| Assay buffer | 50 ml |
| 1000X detection reagent | 17 µl |
| TMB | 12 ml |
| TMB stop solution | 12 ml |
| **Do not** mix or substitute reagents with those from other lots. | |

## Materials and instruments required but not supplied

- Precision pipettes calibrated to deliver 5-1000µL
- Multi-channel pipette calibrated to deliver 50-200µL
- Plate shaker
- Disposable tips
- Vortex-Mixer
- Distilled or de-ionized water
- Microplate reader capable of reading 450nm with background subtraction at 620nm

## Safety precautions

- The test protocol must be followed strictly.
- All reagents containing human material should be handled as if potentially infectious. Operators should wear gloves and protective clothing when handling any patient sera or serum based products.
- The kit reagents contain antimicrobial agents, acid and 3,3',5,5'-tetramethylbenzidine. Avoid contact with the skin and eyes. Rinse immediately with plenty of water if any contact occurs.
- Any liquid that has been brought into contact with potentially infectious material has to be discarded in a container with a disinfectant. Disposal must be performed in accordance with local regulations.
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- Only trained laboratory personnel should execute this test.

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## Preparation of reagents

Prepare only the appropriate amount of required reagent on the day of use. Store all reagents as per instructions stated on the label.

1. **Wash Buffer (1X) Preparation:** Dilute wash buffer concentrate with ultra-pure water 1/20 before use (for example, add 50mL wash buffer concentrate to 450mL ultra-pure water). Mix well.
2. **Detection Reagent (1X) Preparation:** Dilute detection reagent with assay buffer 1/1000 before use (for example add 11µl concentrate to 11ml of assay buffer). Mix well.
3. **Calibrator Preparation:** Dilute the calibrator from 1.15mg/ml down to 11.5µg/ml by pipetting 5µl of calibrator stock into 495µl assay buffer. Label "Cal. Int." Mix well. Prepare calibrators with concentrations ranging from 1440ng/ml to 45ng/ml. The following is an example calibrator curve.

| Sol'n ID | Source | Source Vol (µl) | Cal* Diluent (µl) | Final Vol (µl) | Final Concen-tration (ng/ml) |
|---|---|---|---|---|---|
| **1*** | Cal. Int. (11.5µg/ml) | 62.6 | 437.4 | 500 | 1440 |
| **2*** | 1* | 50 | 50 | 100 | 720 |
| **3*** | 2* | 50 | 50 | 100 | 360 |
| **4*** | 3* | 50 | 50 | 100 | 180 |
| **5*** | 4* | 50 | 50 | 100 | 90 |
| **6*** | 5* | 50 | 50 | 100 | 45 |
| **Neg** | - | - | 100 | 100 | 0 |

1. Remove coated microtiter plate from -20ºC and allow it to acclimate to room temperature for 5-10 minutes.
2. Dilute calibrators and test samples 1/50 with assay buffer (for example add 5µl of prepared calibrator or sample to 245µl of assay buffer). Mix well. Do not store diluted samples.
3. Add 100µL diluted calibrators and samples to appropriate wells on the plate. Incubate for 1 hour at room temperature on a plate shaker at 300rpm.
4. Discard the content of the plate and wash the wells 3x with 200µL wash buffer per well.
5. Add 100µL detection reagent to appropriate wells on the plate. Incubate for 1 hour at room temperature on a plate shaker at approx 300rpm.
6. Discard the content of the plate and wash the wells 3x with 200µL wash buffer per well.
7. 100µL of TMB to each well on plate. Incubate for 5 minutes at room temperature protected from light.
8. Add 100µL of TMB stop solution to each well on plate. Mix by gently tapping the side of the plate.
9. Determine absorbance with a microplate reader at 450nm against 620nm.

## Calculations and results

1. Construct a standard curve by plotting the absorbance obtained from each standard against concentration. Use a 4 or 5 parameter curve fit.
2. The concentration of the unknowns can be back calculated directly from this standard curve using the absorbance value for each sample.