# hGH Pharmacokinetic ELISA
# Catalog EL-1611-878

## For the quantitative determination of hGH in serum and plasma.

## Introduction

The AffinityImmuno GH ELISA kit contains the components required for measuring natural and recombinant human GH in biological matrices such as human serum, plasma or cell culture supernatant.

## Principle of the assay

This assay employs the indirect sandwich enzyme immunoassay technique. Anti-GH is coated onto a 96 well microplate. Calibrator and test samples prepared by dilution into assay buffer and are pipetted into the appropriate wells. GH present in biological matrices is bound by the immobilized anti- GH antibody. After washing away any unbound substances, tagged anti-GH secondary antibody is added to the wells. After washing, diluted detection reagent is added to the wells. The plate is washed to remove any unbound antibody-enzyme reagent and a substrate solution is added to the wells for color development. The color development is proportional to the amount of GH present in 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 (1x8 strips) | 1 |
| Calibrator diluent | 1.8ml |
| Calibrator (1mg/ml) | 12µl |
| 20X wash buffer | 25ml |
| Assay buffer | 50ml |
| 1000X secondary antibody | 17µl |
| 1000X detection reagent | 17µl |
| TMB | 12ml |
| TMB stop solution | 12ml |
| Plate sealers | 3 |
| **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.

## 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 25mL concentrate to 475mL ultra-pure water). Mix well.
2. **Secondary Antibody (1X) Preparation:** Dilute secondary antibody with assay buffer 1/1000 before use (for example add 12µl to 12mL of assay buffer). Mix well.
3. **Detection Reagent (1X) Preparation:** Dilute detection reagent with assay buffer 1/1000 before use (for example add 12µl concentrate to 12ml of assay buffer). Mix well.
4. **Calibrator Preparation:** Dilute the calibrator from 1mg/ml down to 5µg/ml by pipetting 5µL of calibrator stock into 995µL assay buffer. Label "Cal. Int." Mix well. Prepare calibrators with concentrations ranging from 500 ng/ml to 9.375 ng/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. (5µg/ml) | 50 | 450 | 500 | 500 |
| **2*** | 1* | 60 | 40 | 100 | 300 |
| **3*** | 2* | 50 | 50 | 100 | 150 |
| **4*** | 3* | 50 | 50 | 100 | 75 |
| **5*** | 4* | 50 | 50 | 100 | 37.5 |
| **6*** | 5* | 50 | 50 | 100 | 18.75 |
| **7*** | 6* | 50 | 50 | 100 | 9.375 |
| **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 1/100 with assay buffer (for example add 5µL of prepared calibrator or sample to 495µL 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 secondary antibody 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. Add 100µL detection reagent to appropriate wells on the plate. Incubate for 1 hour at room temperature on a plate shaker at approx 300rpm.
8. Discard the content of the plate and wash the wells 3x with 200µL wash buffer per well.
9. Add 100µL of TMB to each well on plate. Incubate for 5 minutes at room temperature protected from light.
10. Add 100µL of TMB stop solution to each well on plate. Mix by gently tapping the side of the plate.
11. 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.
3. Any sample diluted more or less than the standard series will need additional data correction. For example, if the sample is diluted 1/50, then the concentration will be calculated by dividing by 2 due to the calibrators being 2 times more diluted. Similarly, if the sample is diluted 1/500, then the concentration will be calculated by multiplying by a correction factor of 5 due to the calibrators being 5 times more concentrated.

## Performance characteristics

**Precision:** Precision was determined by analyzing samples at 5000 (ULOQ), 4250, 2000, 282 and 93.75 pg/mL (LLOQ) in 6 replicates on 6 different occasions. Intra-assay and Inter-assay coefficient of variation (CV) was < 25% for ULOQ and LLOQ and <20% for the remaining concentrations.

**Accuracy:** Accuracy was determined by analyzing samples at 5000 (ULOQ), 4250, 2000, 282 and 93.75 pg/mL (LLOQ) in 6 replicates on 6 different occasions. Percent error was < 25% for ULOQ and LLOQ and <20% for the remaining concentrations.

**Detection Limit:** The detection limit is 93.75 pg/mL.

**Specificity:** In presence of interfering materials, prolactin, IGF-I,IGF-II, M-CSF, GM-CSF, IGFRB-3 and IL-3RB, all levels of hGH met the acceptance criteria of having a percent error of ± 20% of concentrations of 3000 ng/mL, 1500 ng/mL, 750 ng/mL, 375 ng/mL and 187.5 ng/mL and a percent error of ± 25% of concentrations of 5000 ng/mL (ULOQ) and 93.75 ng/mL (LLOQ).

## Ordering Information

Please vist www.affinityimmuno.com to order this product. Visa, Mastercard, AMEX and PayPal are accepted in our online store.

Your order will be processed immediately and you will be notified with a delivery timeframe.