Product Manual

Human Growth Hormone ELISA kit

Catalog #: ENZ-KIT148-0001

96-Well Kit

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Product Manual

Please read entire booklet before proceeding with the assay.

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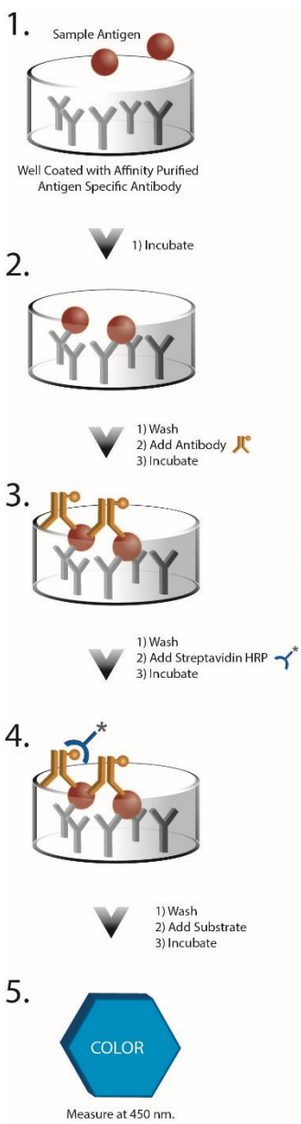
INTRODUCTION

The Human Growth Hormone (HGH, Somatotropin) ELISA kit is a complete kit for the quantitative determination of HGH in human serum, human plasma, human saliva, human urine, canine serum and tissue culture media. Please read the complete kit insert before performing this assay.

Growth hormone is produced by the pituitary gland to fuel childhood growth and help maintain tissues and organs throughout life. HGH is a 22,000 dalton protein made up of 191 amino acids. Growth hormone initiates a wide variety of growth promoting pathways including the MAPK/ERK pathway resulting in direct stimulation and outgrowth of chondrocytes of cartilage as well as the JAK/STAT pathway resulting in IGF-1 production which promotes growth in a wide variety of tissues[1]. Additionally HGH is known to increase calcium retention, increase muscle mass, promote lipolysis, increase protein synthesis, stimulate the growth of all internal organs, increase glucose availability and stimulate the immune system. The broad spectrum of tissues impacted by HGH lend to its use in the treatment of diverse conditions including growth disorders, obesity, bone disease, diabetes, cardiovascular disease, memory/cognitive disorders and diseases of aging [2,3,4,5,6]. The anabolic effects of HGH have resulted in its use and abuse by athletes. In addition to its potential for physical performance enhancement, there is considerable interest in the use of HGH in anti-aging and cosmetic applications.



PRINCIPLE

1. The kit uses a monoclonal antibody to HGH immobilized on a microtiter plate. Standards or samples containing HGH are added to the plate.
2. The plate is then incubated. During this incubation the HGH antibody immobilized on the plate binds HGH in the standards or samples.
3. The plate is washed, removing excess unbound sample or standard. A solution of biotinylated monoclonal antibody to HGH is then added. This antibody binds to the HGH captured on the plate.
4. After a short incubation the plate is washed to remove unbound biotinylated antibody. Streptavidin conjugated to horseradish peroxidase (SA-HRP) is added and the plate is then incubated.
5. The plate is washed to remove excess conjugate. TMB substrate solution is added to all wells and incubated. An HRP-catalyzed reaction generates a blue color in the solution.
6. Stop solution is added to stop the substrate reaction. The resulting yellow color is read at 450nm. The amount of signal is directly proportional to the level of HGH in the sample or standard.

MATERIALS SUPPLIED

Do not mix components from different kit lots or use beyond the expiration date of the kit.

1. **HGH Microtiter Plate, One Plate of 96 Wells**
   Catalog No. 80-2711

   A plate using break-apart strips coated with a monoclonal antibody specific to HGH

2. **HGH Standard, 5 ng/mL**
   Catalog No. 80-2710

   One vial containing 125 µL of 5 ng/ml recombinant HGH

3. **HGH Detector Antibody, Lyophilized**
   Catalog No. 80-2712

   Lyophilized biotinylated HGH monoclonal antibody

4. **Antibody Diluent, 14 mL**
   Catalog No. 80-2722

   A yellow solution of Tris buffered saline containing BSA and detergents

5. **HGH Conjugate, 10 mL**
   Catalog No. 80-2713

   A blue solution of Streptavidin conjugated to horseradish peroxidase

Protect substrate from prolonged exposure to light.

6. **Assay Buffer 13, 60 mL**
   Catalog No. 80-1500

   Tris buffered saline containing BSA and detergents

7. **TMB Substrate, 10 mL**
   Catalog No. 80-0350

   A solution of 3,3’5,5’ tetramethylbenzidine (TMB) and hydrogen peroxide

Stop solution is caustic. Keep tightly capped.

8. **Stop Solution 2, 10 mL**
   Catalog No. 80-0377

   One bottle containing 1N hydrochloric acid in water

9. **Wash Buffer Concentrate, 100 mL**
   Catalog No. 80-1287

   One bottle containing 20x Tris buffered saline containing detergent

10. **HGH Assay Layout Sheet**
    Catalog No. 30-0335

11. **Plate Sealer, 3 each**
    Catalog No. 30-0012

STORAGE

All kit components are stable at 4°C until the kit's expiration date. Shipping conditions may not reflect storage conditions.

OTHER MATERIALS NEEDED

1. Deionized or distilled water
2. Precision pipets for volumes between 5 µL and 1,000 µL
3. Repeater pipet for dispensing 100 µL
4. Disposable beakers for diluting buffer concentrates
5. Graduated cylinders
6. A microplate shaker
7. Adsorbent paper for blotting
8. Microplate reader capable of reading a 450nm
9. Software for extrapolating sample values from optical density readings utilizing a four parameter logistic curve fit.

If buffers other than those provided are used, the end-user must determine the appropriate dilution and assay variation.

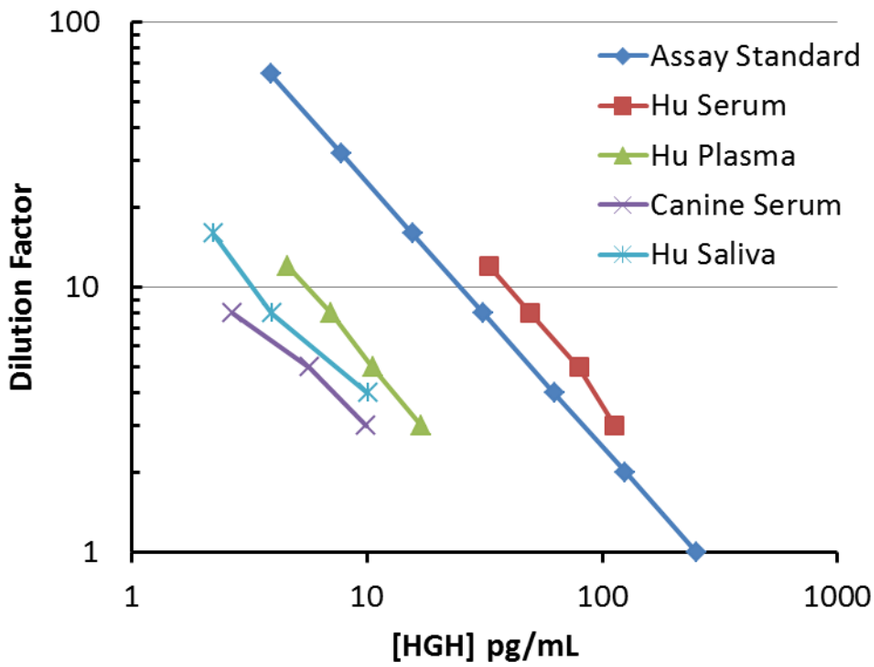
SAMPLE HANDLING

The Human Growth Hormone ELISA is compatible with human serum, human plasma, human saliva, canine serum and tissue culture media. Samples diluted sufficiently into Assay Buffer 13 can be read directly from a standard curve. The recommended minimal dilution to remove matrix interference for human serum, plasma and saliva samples is 1:4 with Assay Buffer 13. Tissue culture media and urine can be tested diluted 1:2 with Assay Buffer 13. The minimal recommended dilution may not be optimal for all samples as the levels of endogenous HGH can vary between sample groups. Therefore, it is up to each end user to optimize the dilution for their unique set of samples.

SAMPLE MATRIX PROPERTIES

Parallelism and Dilutional Linearity

The parallelism of various matrices was determined by running serial dilutions in the assay, assigning concentrations to each dilution and plotting the dilution factor against the determined concentration of each matrix dilution. Parallelism of the curves demonstrates that the antigen binding characteristics are similar enough to allow the accurate determination of native analyte levels in diluted samples of human and canine origin.



Dilutional linearity in matrix dilutions is achieved when returned values approximately match after multiplying them by the dilution factor. Linearity was achieved at 1:3 for human serum, human plasma and canine serum and at 1:4 human saliva.

Dilutional Linearity			
Dilution Factor	Human Serum	Human EDTA Plasma	Canine Serum
3	100%	100%	100%
5	118%	104%	96%
8	117%	110%	72%
12	118%	108%	113%

Dilutional Linearity	
Dilution Factor	Human Saliva
4	100%
8	79%
16	88%

Spike and Recovery

HGH was spiked at three concentrations into diluted human serum, EDTA plasma, urine, saliva and tissue culture media. Matrix background was subtracted and the percent recovery of the spiked HGH was determined.

Sample Matrix	Dilution	Spike Concentration (pg/mL)	% Recovery of Spike
Human Serum	1:4	200	99
		100	101
		50	91
Human Plasma	1:4	200	97
		100	82
		50	119
Human Urine	1:2	200	106
		100	120
		50	117
Tissue Culture Media	1:2	200	88
		100	92
		50	87
Human Saliva	1:4	125	88

REAGENT PREPARATION

Sample handling procedures should be completed prior to reagent preparation.

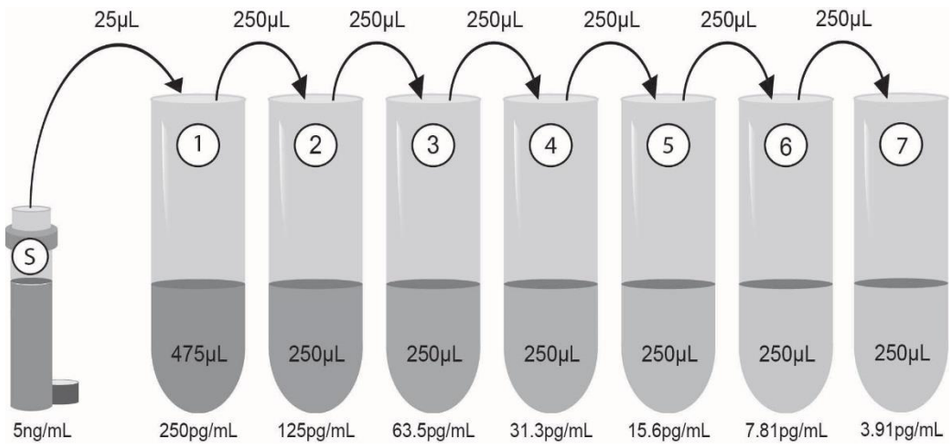
Polypropylene tubes may be used for standard preparation. Avoid polystyrene.

1. Wash Buffer

Prepare Wash Buffer by diluting 50 mL of the supplied concentrate with 950 mL of deionized water. Store the diluted wash buffer at room temperature. Diluted wash buffer should be used within 3 months.

2. HGH Standard

Allow the HGH standard to warm to room temperature. Label seven 12x75 mm polypropylene tubes #1 through #7. Add 475 µL of Assay Buffer 13 into tube #1. Add 250 µL of Assay Buffer 13 into tube #2 through tube #7. Add 25 µL of 5 ng/mL HGH standard stock to tube #1 and vortex. Add 250 µL of tube #1 into tube #2 and vortex. Add 250 µL of tube #2 to tube #3 and vortex thoroughly. Continue this for tubes #4 through #7.



Diluted standards should be used within 60 minutes of preparation. Discard any unused standard dilutions.

All other kit components should be brought to room temperature prior to use in the assay.

3. HGH Detector Antibody

Reconstitute the lyophilized HGH Detector Antibody in 12 mL of the yellow Antibody Diluent. Lyophilized antibody may have dislodged from the bottom of the vial during shipping, lightly vortex the vial to assure complete reconstitution. Store any unused reconstituted antibody at -20°C. Allow no more than three freeze-thaw cycles.

ASSAY PROCEDURE

Bring all reagents to room temperature for at least 30 minutes prior to opening.

Refer to the Assay Layout Sheet to determine the number of wells to be used. Remove the wells not needed for the assay and return them, with the desiccant, to the mylar bag and seal. Store unused wells at 4°C.

All standards and samples should be run in duplicate.

1. Add 100 µL of Assay Buffer 13 into the S0 (0pg/ml standard) and NSB wells. Leave the Blank and TA wells empty.
2. Add 100 µL of standards #1 through #7 into the appropriate wells.
3. Add 100 µL of the samples into the appropriate wells.
4. Seal the plate and incubate at room temperature (RT) on a plate shaker for 1 hr at ~ 500 rpm*. **See note.**
5. Empty the contents of the wells and wash by adding a full well volume ~400 µL of 1X Wash Buffer to each well. Empty or aspirate the wells and repeat the wash 3 more times for a total of 4 washes. After the final wash, empty or aspirate the wells and firmly tap the plate on a lint free paper towel to remove any remaining wash buffer.

Add the reagents to the sides of the wells to avoid possible contamination.

6. Add 100 µL of reconstituted Detector Antibody (yellow) into each well, except the NSB, TA and Blank wells. Add 100 µL Assay Buffer 13 into NSB wells and leave Blank and TA wells empty.
7. Seal the plate and incubate for 30 minutes with shaking on a plate shaker at room temperature.
8. Wash as above (Step 5).
9. Add 100 µL of HGH SA-HRP Conjugate (blue) to each well, except the Blank.
10. Seal the plate and incubate for 30 minutes with mixing on a plate shaker at room temperature
11. Wash as above (Step 5).
12. Dilute HGH SA-HRP Conjugate 1:100 with Assay Buffer 13 and add 5 µL of this dilution to TA wells.
13. Add 100 µL of TMB solution into all wells.
14. Seal the plate. Incubate for 30 minutes with shaking on a plate shaker at room temperature.
15. Add 100 µL of Stop Solution into each well.
16. After blanking the plate reader against the substrate, read optical density at 450nm. If the plate reader is not capable of adjusting for the blank, manually subtract the mean OD of the substrate blank from all readings.

*** Note: The plate shaker speed was based on a BellCo Mini Orbital Shaker (mod no. 7744-08096). The actual speed of the plate shaker should be such that the liquid in the plate wells mixes thoroughly, but does not splash out of the well.**

CALCULATION OF RESULTS

Be sure to multiply sample concentrations by the dilution factor used during sample preparation.

Several options are available for the calculation of the concentration of HGH in the samples. We recommend that the data be handled by an immunoassay software package utilizing a 4 parameter logistic (4PL) curve fitting program. The concentration of HGH can be calculated as follows:

1. Calculate the average net OD for each standard and sample by subtracting the average NSB OD from the average OD for each standard and sample.

   Average Net OD = Average OD - Average NSB OD

2. Using data analysis software, plot the Average Net OD for each standard versus HGH concentration in each standard.

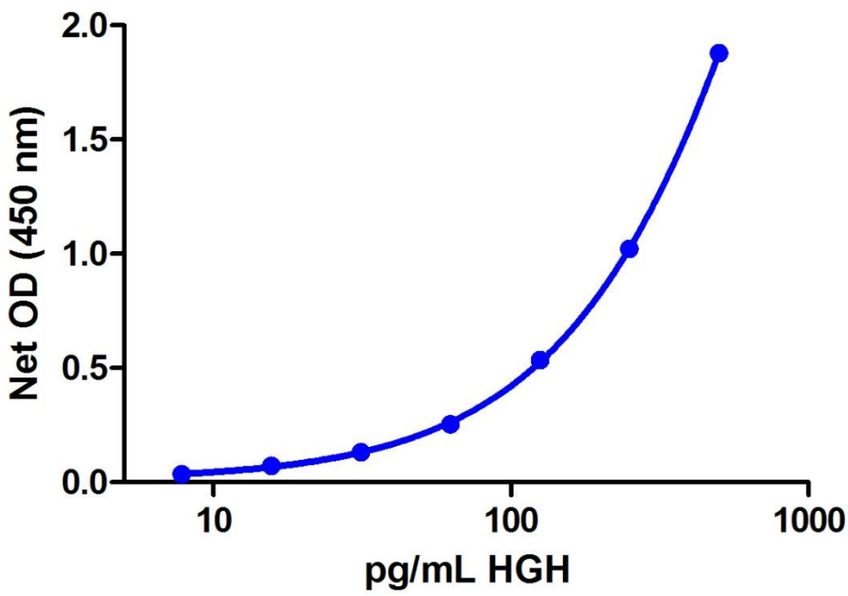
TYPICAL RESULTS

The results shown below are for illustration only and should not be used to calculate results.

Typical Results			
Sample	**Mean OD**	**Net OD**	**HGH (pg/mL)**
NSB	0.057	--	--
S1	1.936	1.879	**250**
S2	1.079	1.022	**125**
S3	0.592	0.535	**62.5**
S4	0.312	0.254	**31.3**
S5	0.191	0.133	**15.6**
S6	0.129	0.072	**7.81**
S7	0.093	0.035	**3.91**
S0	0.066	0.009	**0**

TYPICAL STANDARD CURVE

Typical standard curves are shown below. These curves must not be used to calculate HGH concentrations; each user must run a standard curve for each assay.



PERFORMANCE CHARACTERISTICS

Specificity

The specificity of the assay was determined by running serial dilutions of the analytes, including the cross-reactant, in the assay, fitting the resulting dose response curve(s) to a 4PL curve-fit and determining the ED50. The ED50 of the standard curve was then divided by the determined ED50 of the cross-reactant and multiplied by 100.

Analyte	Cross Reactivity
Prolactin	≤ 0.16%
Human placental lactogen (hPL), also called human chorionic somatomammotropin	≤ 0.16%
Rat Growth Hormone	<0.16%
Mouse Growth Hormone	<1.5%

Sensitivity

The sensitivity or limit of detection of the assay is 0.93 pg/mL as determined by interpolation at 2 standard deviations above the background (0 pg/mL) of 12 zero standard replicates. The results from 6 separate standard curves were averaged for this determination.

Interference

Protease Inhibitor Cocktail (PIC) and PMSF are commonly used protease inhibitors and may be used in the collection of clinical specimens to be tested for HGH if needed. The typical concentration of PIC and PMSF added to specimens is 0.5% and 0.1 mM, respectively. To determine the level of interference, each interfering substance was serially diluted into assay buffer, each dilution was spiked with a constant concentration of HGH (62.5pg/ml) and each sample was run in the assay. This data suggests that the assay can accurately detect HGH in the presence of protease inhibitors.

Protease inhibitor	Assay Tolerance
PIC	<1.25%
PMSF	<2.5 mM

Intra-assay precision was determined by assaying 20 replicates of three matrix controls containing HGH in a single assay.

Intra-assay precision	
pg/mL	**%CV**
128.6	4.0
67.5	4.2
34.5	4.0

Inter-assay precision was determined by measuring matrix controls of varying HGH concentrations in multiple assays over several days.

Inter-assay precision	
pg/mL	**%CV**
136.1	6.2
73.2	6.4
37.9	1.9

REFERENCES

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GLOBAL HEADQUARTERS
Enzo Life Sciences Inc.
10 Executive Boulevard
Farmingdale, NY 11735
Toll-Free:1.800.942.0430
Phone:631.694.7070
Fax: 631.694.7501
info-usa@enzolifesciences.com

EUROPE
Enzo Life Sciences (ELS) AG
Industriestrasse 17
CH-4415 Lausen
Switzerland
Phone:+41/0 61 926 89 89
Fax:+41/0 61 926 89 79
info-ch@enzolifesciences.com

For local distributors and detailed product information visit us online:
www.enzolifesciences.com