



**PORCINE / CANINE GROWTH HORMONE RIA KIT  
125 TUBES (Cat. # PGH-46HK)**

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**I. INTENDED USE**

Growth Hormone is secreted from the anterior pituitary and its measurement in circulation reflects pituitary function particularly of somatotrophs. Growth Hormone influences both whole body metabolism and growth. This Porcine / Canine Growth Hormone radioimmunoassay has been developed to quantitate Growth Hormone in plasma, serum, and tissue culture media. It is a completely homologous assay since the antibody was raised against recombinant Porcine Growth Hormone and both the standard and tracer are prepared with recombinant Porcine Growth Hormone. Since the amino acid sequences of Porcine Growth Hormone and Canine Growth Hormone are identical, this assay developed for Porcine Growth Hormone measures Canine Growth Hormone levels with equal efficiency. ***This kit is for research purposes only.***

**II. PRINCIPLES OF PROCEDURE**

In radioimmunoassay, a fixed concentration of labeled tracer antigen is incubated with a constant dilution of antiserum such that the concentration of antigen binding sites on the antibody is limited, for example, only 50% of the total tracer concentration may be bound by antibody. If unlabeled antigen is added to this system, there is competition between labeled tracer and unlabeled antigen for the limited and constant number of binding sites on the antibody. Thus, the amount of tracer bound to antibody will decrease as the concentration of unlabeled antigen increases. This can be measured after separating antibody-bound from free tracer and counting one or the other, or both fractions. A calibration or standard curve is set up with increasing concentrations of standard unlabeled antigen and from this curve the amount of antigen in unknown samples can be calculated. Thus, the four basic necessities for a radioimmunoassay system are: a specific antiserum to the antigen to be measured, the availability of a radioactive labeled form of the antigen, a method whereby antibody-bound tracer can be separated from the unbound tracer, and finally, an instrument to count radioactivity.<sup>1</sup>

The Millipore Porcine / Canine Growth Hormone assay utilizes <sup>125</sup>I-labeled Porcine Growth Hormone and a Porcine Growth Hormone antiserum to determine the level of Porcine or Canine Growth Hormone in serum, plasma or tissue culture media by the double antibody/PEG technique.

**III. REAGENTS SUPPLIED**

Each kit is sufficient to run 125 tubes and contains the following reagents.

**A. Assay Buffer**

0.05M Phosphosaline pH 7.4 containing 0.025M EDTA, 0.08% Sodium Azide, 1% RIA Grade BSA  
Quantity: 20 mL/vial  
Preparation: Ready to use

**B. Antiserum**

Guinea Pig anti-Porcine Growth Hormone Serum in Assay Buffer  
Quantity: 13 mL/vial  
Preparation: Ready to use

### III. REAGENTS SUPPLIED (continued)

#### C. <sup>125</sup>I-Porcine / Canine Growth Hormone

<sup>125</sup>I-Porcine Growth Hormone Label, (specific activity 98 μCi/μg)

Lyophilized for stability. Freshly iodinated label contains < 1.5 μCi (< 56 kBq), calibrated to the 1st Monday of each month.

Quantity: 13.5 mL/vial upon hydration

Preparation: Contents Lyophilized. Hydrate with entire contents of Label Hydrating Buffer. Allow to set at room temperature for 30 minutes, with occasional gentle mixing.

#### D. Label Hydrating Buffer

Assay Buffer containing Normal Guinea Pig Serum as a carrier. Used to hydrate <sup>125</sup>I-Porcine / Canine GH.

Quantity: 13.5 mL/vial

Preparation: Ready to use

#### E. Standards

Recombinant Porcine Growth Hormone in Assay Buffer at the following concentrations:

1, 2, 5, 10, 20, 50, 100 ng/mL

Quantity: 1 mL/vial

Preparation: Ready to use

#### F. Quality Controls 1 & 2

Recombinant Porcine Growth Hormone in Assay Buffer

Quantity: 1 mL/vial

Preparation: Ready to use

#### G. Precipitating Reagent

Goat anti Guinea Pig IgG Serum, 3% PEG and 0.05% Triton X-100 in 0.05M Phosphosaline, 0.025M EDTA,

0.08% Sodium Azide

Quantity: 130 mL/vial

Preparation: Ready to use; chill to 4°C.

### IV. STORAGE AND STABILITY

Refrigerate all reagents between 2 and 8°C for short term storage. For prolonged storage (>2 weeks), freeze at ≤ -20°C. Avoid multiple (> 2) freeze/thaw cycles. Refer to date on bottle for expiration when stored at ≤ -20°C. Do not mix reagents from different kits unless they have the same lot number.

### V. REAGENT PRECAUTIONS

#### A. Radioactive Materials

This radioactive material may be received, acquired, possessed and used only by research personnel or clinical laboratories for in vitro research tests not involving internal or external administration of the material, or the radiation therefrom, to human beings or animals. Its receipt, acquisition, possession, use and transfer are subject to the regulations of the U. S. Nuclear Regulatory Commission (NRC) or of a State with which the Commission has entered into an agreement for the exercise of regulatory authority.

The following are suggested general rules for the safe use of radioactive material. The customer's Radiation Safety Officer (RSO) is ultimately responsible for the safe handling and use of radioactive material.

## V. REAGENT PRECAUTIONS (continued)

1. Wear appropriate personal devices at all times while in areas where radioactive materials are used or stored.
2. Wear laboratory coats, disposable gloves, and other protective clothing at all times.
3. Monitor hands, shoes, and clothing and immediate area surrounding the work station for contamination after each procedure and before leaving the area.
4. Do not eat, drink, or smoke in any area where radioactive materials are stored or used.
5. Never pipette radioactive material by mouth.
6. Dispose of radioactive waste in accordance with NRC rules and regulations.
7. Avoid contaminating objects such as telephones, light switches, doorknobs, etc.
8. Use absorbent pads for containing and easily disposing of small amounts of contamination.
9. Wipe up all spills immediately and thoroughly and dispose of the contaminated materials as radioactive waste. Inform RSO.

### B. Sodium Azide

Sodium Azide has been added to all reagents as a preservative at a concentration of 0.08%. Although it is at a minimum concentration, sodium azide may react with lead and copper plumbing to form highly explosive metal azides. On disposal, flush with a large volume of water to prevent azide build up.

## VI. MATERIALS REQUIRED BUT NOT PROVIDED

1. Borosilicate glass tubes, 12 x 75 mm. (NOTE: Polypropylene or polystyrene tubes may be used if the investigator finds that the pellet formation is acceptably stable in their system.)
2. 100  $\mu$ L pipet with disposable tips
3. 100  $\mu$ L & 1.0 mL repeating dispenser
4. Refrigerated swing bucket centrifuge capable of developing 2,000 - 3,000 xg. (Use of fixed-angle buckets are not recommended.)
5. Absorbent paper
6. Vortex mixer
7. Refrigerator
8. Gamma Counter

## VII. SPECIMEN COLLECTION AND STORAGE

1. A maximum of 100  $\mu$ L per assay tube of serum or plasma can be used, although, 50  $\mu$ L per assay tube is adequate for most applications. Tissue culture and other media may also be used.
2. Care must be taken when using heparin as an anticoagulant, since an excess will provide falsely high values.<sup>2</sup>  
Use no more than 10 IU heparin per mL of blood collected.
3. Specimens can be stored at 4°C if they will be tested within 24 hours of collection. For longer storage, specimens should be stored at  $\leq$  -20°C. Avoid multiple (> 2) freeze/thaw cycles.
4. Avoid using samples with gross hemolysis or lipemia.

## VIII. ASSAY PROCEDURE

For optimal results, accurate pipetting and adherence to the protocol are recommended.

### A. Assay Set-Up, Day One

1. Pipet 300  $\mu$ L of Assay Buffer to the Non-Specific Binding (NSB) tubes (3-4) and 200  $\mu$ L to Reference (Bo) tubes (5-6). Pipette 100  $\mu$ L of Assay Buffer to tubes seven through the end of the assay.
2. Pipet 100  $\mu$ L of Standards and Quality Controls in duplicate (see flow chart).
3. Pipet 100  $\mu$ L of each sample in duplicate. (NOTE: Smaller volumes of sample may be used when Porcine / Canine Growth Hormone concentrations are anticipated to be elevated or when sample size is limited. Additional Assay Buffer should be added to compensate for the difference so that the volume is equivalent to 100  $\mu$ L, e.g. when using 50  $\mu$ L of sample, add 50  $\mu$ L of Assay Buffer). Refer to Section IX for calculation modification.
4. Pipet 100  $\mu$ L of  $^{125}$ I-Porcine / Canine Growth Hormone to all tubes. Important: For preparation, see Section III, Part C.
5. Pipet 100  $\mu$ L of GP Porcine / Canine antiserum to all tubes except Total Count tubes (1-2) and NSB tubes (3-4).
6. Vortex, cover, and incubate overnight (20-24 hours) at room temperature.

### B. Day Two

7. Add 1.0 mL of cold (4°C) Precipitating Reagent to all tubes (except Total Count tubes).
8. Vortex and incubate 20 minutes at 4°C.
9. Centrifuge, 4°C, all tubes [except Total Count tubes (1-2)] for 20 minutes at 2,000-3,000 xg. NOTE: If less than 2,000 xg is used or if slipped pellets have been observed in previous runs, the time of centrifugation must be increased to obtain a firm pellet (e.g., 40 minutes). Multiple centrifuge runs within an assay must be consistent.

Conversion of rpm to xg:

$$xg = (1.12 \times 10^{-5}) (r) (\text{rpm})^2$$

r = radial distance in cm (from axis of rotation to the bottom of the tube)

rpm = revolutions per minute

10. Immediately decant the supernate of all tubes except Total Count tubes (1-2), drain tubes for at least 15-60 seconds (be consistent between racks), and blot excess liquid from lip of tubes. NOTE: Invert tubes only one time. Pellets are fragile and slipping may occur.
11. Count all tubes in a gamma counter for 1 minute. Calculate the ng/mL of Porcine / Canine Growth Hormone in unknown samples using automated data reduction procedures.

## VIII. ASSAY PROCEDURE (continued)

### Assay Procedure Flow Chart

Day One						Day Two		
Set-up	Step 1	Steps 2 & 3	Step 4	Step 5	Step 6	Step 7	Step 8	Steps 9-11
Tube Number	Add Assay Buffer	Add Standard / QC Sample	Add I-125 Porcine / Canine GH Tracer	Add GP Porcine/ Canine GP Antiserum	<b>Vortex, Cover, and Incubate 20-24 hrs at RT</b>	Add Precipitating Reagent	<b>Vortex, cover, and Incubate 20 min. at 4°C</b>	<b>Centrifuge at 4°C for 20 min., Decant, and Count</b>
1,2	-	Total Count	100 µl	-	↓	-	↓	↓
3,4	300 µl	NSB	100 µl	-		1.0 mL		
5,6	200 µl	Reference (Bo)	100 µl	100 µl		1.0 mL		
7,8	100 µl	100 µl of 1 ng/mL	100 µl	100 µl		1.0 mL		
9,10	100 µl	100 µl of 2 ng/mL	100 µl	100 µl		1.0 mL		
11,12	100 µl	100 µl of 5 ng/mL	100 µl	100 µl		1.0 mL		
13,14	100 µl	100 µl of 10 ng/mL	100 µl	100 µl		1.0 mL		
15,16	100 µl	100 µl of 20 ng/mL	100 µl	100 µl		1.0 mL		
17,18	100 µl	100 µl of 50 ng/mL	100 µl	100 µl		1.0 mL		
19,20	100 µl	100 µl of 100 ng/mL	100 µl	100 µl		1.0 mL		
21,22	100 µl	100 µl of QC 1	100 µl	100 µl		1.0 mL		
23,24	100 µl	100 µl of QC 2	100 µl	100 µl		1.0 mL		
25,n	100 µl	100 µl of unknown	100 µl	100 µl		1.0 mL		

## IX. CALCULATIONS

### A. Explanation

The calculations for Porcine / Canine Growth Hormone can be automatically performed by most gamma counters possessing data reduction capabilities or by independent treatment of the raw data using a commercially available software package. Choose weighted 4-parameter or weighted log/logit for the mathematical treatment of the data. [NOTE: Be certain the procedure used subtracts the NSB counts from each average count, except Total Counts, prior to final data reduction.]

### B. Manual Calculation

1. Average duplicate counts for Total Count tubes (1-2), NSB tubes (3-4), Total Binding tubes (reference, Bo) (5-6), and all duplicate tubes for standards and samples to the end of the assay.
2. Subtract the average NSB counts from each average count (except for Total Counts). These counts are used in the following calculations.
3. Calculate the percentage of tracer bound  
(Total Binding Counts/Total Counts) X 100  
This should be 25-45%.
4. Calculate the percentage of total binding (%B/Bo) for each standard and sample  
$$\%B/Bo = (\text{Sample or Standard/Total Binding}) \times 100$$
5. Plot the % B/Bo for each standard on the y-axis and the known concentration of the standard on the x-axis using log-log graph paper.
6. Construct the reference curve by joining the points with a smooth curve.
7. Determine the ng/mL of Porcine / Canine Growth Hormone in the unknown samples and controls by interpolation of the reference curve.

## IX. CALCULATIONS (continued)

NOTE: When sample volumes assayed differ from 100  $\mu\text{L}$ , an appropriate mathematical adjustment must be made to accommodate for the dilution factor (e.g., if 50  $\mu\text{L}$  of sample is used, then calculated data must be multiplied by 2).

## X. INTERPRETATION

### A. Acceptance Criteria

1. The run will be considered accepted when all Quality Control values fall within the calculated Quality Control Range; if any QC's fall outside the control range, review results with the supervisor.
2. If the difference between duplicate results of a sample is  $>10\%$  CV, repeat the sample.
3. The limit of sensitivity for the Porcine / Canine Growth Hormone assay is 1 ng/mL (100  $\mu\text{L}$  sample size).
4. The limit of linearity for the Porcine / Canine Growth Hormone assay is 100 ng/mL (100  $\mu\text{L}$  sample size). Any result greater than 100 ng/mL should be repeated on dilution using Assay Buffer as a diluent.

## XI. NORMAL FASTING RANGE

To Be Determined

## XII. ASSAY CHARACTERISTICS

### A. Sensitivity

The lowest level of Porcine / Canine Growth Hormone that can be detected by this assay is 1 ng/mL when using a 100  $\mu\text{L}$  sample size.

### B. Performance

The following parameters of assay performance are expressed as Mean  $\pm$  Standard Deviation.

$$\text{ED}_{80} = 2.5 \pm 0.3 \text{ ng/mL}$$

$$\text{ED}_{50} = 9.1 \pm 0.7 \text{ ng/mL}$$

$$\text{ED}_{20} = 34.8 \pm 3.9 \text{ ng/mL}$$

### C. Specificity

The specificity (also known as selectivity) of an analytical test is its ability to selectively measure the analyte in the presence of other like components in the sample matrix.

Porcine Prolactin	$<0.1\%$
Human Growth Hormone	$<0.5\%$

Rat Growth Hormone	The cross-reactivity pattern of Rat Growth Hormone in Porcine / Canine Growth Hormone RIA is biphasic. Up to 10.0 ng/mL concentration of RGH, the cross-reactivity is 100%. At higher concentrations of RGH, the cross-reactivity progressively decreases. It is $\sim 1.0\%$ at 5 $\mu\text{g/mL}$ .
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## XII. ASSAY CHARACTERISTICS (continued)

### D. Precision

Within and Between Assay Variation

Sample Number	Mean ng/mL	Within % CV	Between % CV
1	4.22	6.27	10.67
2	36.75	5.36	8.18

Within and between assay variations were performed on two samples containing varying concentrations of Porcine/Canine Growth Hormone. Data (mean and % CV) shown are from one assay with twelve determinations of each sample for intra-assay precision. For inter-assay precision, data are generated using twelve separate assays run for the two samples in quadruplicate.

### E. Recovery

Spike and Recovery of Porcine Growth Hormone in Dog Serum

Porcine GH Added ng/mL	Observed ng/mL	Expected ng/mL	% Recovery
0	1.5	1.5	100
2.5	3.5	4.0	88
5	7.0	6.5	108
25	30.9	26.5	117

Varying concentrations of Porcine Growth Hormone were added to Dog serum and the Porcine Growth Hormone content was determined by RIA. Mean of the observed levels from five duplicate determinations in three separate assays are shown. Percent recovery was calculated on the observed vs. expected.

## XII. ASSAY CHARACTERISTICS (continued)

### F. Linearity

Effect of Serum Dilution

Sample #	Volume Sampled	Observed ng/mL	Expected ng/mL	% Of Expected
1	100 µl	21.1	21.1	100
	75 µl	21.0		100
	50 µl	18.7		89
	25 µl	17.2		82
2	100 µl	4.0	4.0	100
	75 µl	4.2		105
	50 µl	4.2		105
	25 µl	4.8		120
3	100 µl	18.3	18.3	100
	75 µl	18.6		102
	50 µl	17.4		95
	25 µl	14.5		79
4	100 µl	40.7	40.7	100
	75 µl	39.8		98
	50 µl	41.1		101
	25 µl	38.1		94
5	100 µl	6.8	6.8	100
	75 µl	6.3		93
	50 µl	5.5		81
	25 µl	4.5		66

Aliquots of Porcine, Canine, or Horse serum containing varying concentrations of Porcine Growth Hormone were analyzed in the volumes indicated. Dilution factors of 1, 1.33, 2 and 4 representing 100 µL, 75 µL, 50 µL and 25 µL, respectively, were applied in calculating observed concentrations. Mean observed Porcine Growth Hormone levels from five duplicate determinations in three separate assays are shown. Percent of expected was calculated on the observed vs. the expected.

**XII. ASSAY CHARACTERISTICS (continued)**

**G. Example of Assay Results**

This data is presented as an example only and should not be used in lieu of a standard curve prepared with each assay.

Tube #	ID	CPM	Ave CPM	Ave Net CPM	% B/Bo	ng/mL
1	Totals	18878				
2	"	18610	18744	-	-	-
3	NSB	468				
4	"	466	467	-	-	-
5	Bo	8023				
6	"	7994	8009	7542	-	-
<u>Standards</u>						
7	1 ng/mL	7028				
8		6991	7010	6543	86.8	-
9	2 ng/mL	6722				
10		6590	6656	6189	82.1	-
11	5 ng/mL	5181				
12		5261	5221	4754	63.0	-
13	10 ng/mL	4067				
14		3940	4004	3537	46.9	-
15	20 ng/mL	3087				
16		2876	2982	2515	33.3	-
17	50 ng/mL	1746				
18		1796	1771	1304	17.3	-
19	100 ng/mL	1291				
20		1211	1251	784	10.4	-
<u>Controls/Unknown</u>						
21	QC 1	5953				
22		5822	5888	5421	71.9	3.32
23	QC 2	2050				
24		2037	2044	1577	20.9	38.76
25-n	Unknown					

### XIII. QUALITY CONTROLS

Good Laboratory Practice (GLP) requires that Quality Control (QC) specimens be run with each standard curve to check the assay performance. Two levels of controls are provided for this purpose. These and any other control materials should be assayed repeatedly to establish mean values and acceptable ranges. Each individual laboratory is responsible for defining their system for Quality Control decisions and is also responsible for making this system a written part of their laboratory manual. The ranges for Quality Control 1 and 2 are provided on the card insert or can be located at the Millipore website [www.millipore.com/bmia](http://www.millipore.com/bmia).

Recommended batch analysis decision using two controls (Westgard Rule)<sup>4</sup>:

1. When both controls are within  $\pm 2$  SD.  
Decision: Approve batch and release analytical results.
2. When one control is outside  $\pm 2$  SD and the second control is within  $\pm 2$  SD.  
Decision: Hold results, check with supervisor. If no obvious source of error is identified by the below mentioned check of systems, the supervisor may decide to release the results.

Technician check of systems:

1. Check for calculation errors
2. Repeat standards and controls
3. Check reagent solutions
4. Check instrument

### XIV. REPLACEMENT REAGENTS

Reagents	Cat. #
<sup>125</sup> I-Porcine/Canine GH (<1.5 $\mu$ Ci, 56 kBq)	9046-HK
Label Hydrating Buffer (13.5mL)	LHB-45HK
Porcine / Canine GH Standards (1 mL each)	8046-K
GP Porcine / Canine GH Antiserum (13 mL)	1046-HK
Precipitating Reagent (130 mL)	PR-UVHK
QC 1&2 (1 mL each)	6046-K
Assay Buffer (20 mL)	AB-PHK

## XV. ORDERING INFORMATION

### A. To place an order:

#### For USA Customers:

Please provide the following information to our customer service department to expedite your telephone, fax or mail order:

1. Your name, telephone and/or fax number
2. Customer account number
3. Shipping and billing address
4. Purchase order number
5. Catalog number and description of product
6. Quantity and product size

NOTE: Appropriate license from NRC (or equivalent) must be on file at Millipore before radioactive orders can be shipped.

#### TELEPHONE ORDERS:

Toll Free US (866) 441-8400  
(636) 441-8400

FAX ORDERS: (636) 441-8050

MAIL ORDERS: Millipore  
6 Research Park Drive  
St. Charles, Missouri 63304 U.S.A.

#### For International Customers:

To best serve our international customers, it is Millipore's policy to sell our products through a network of distributors. To place an order or to obtain additional information about Millipore products, please contact your local distributor.

### B. Conditions of Sale

All products are for research or manufacturing use only. They are not intended for use in clinical diagnosis or for administration to humans or animals. All products are intended for *in vitro* use only.

### C. Material Safety Data Sheets (MSDS)

Material safety data sheets for Millipore products may be ordered by fax or phone. See Section A above for details on ordering.

## XVI. REFERENCES

1. Morgan, C.R. and Lazarow, A. Immunoassay of Insulin: Two antibody system. Plasma insulin levels in normal, Subdiabetic, and diabetic rats. *Diabetes* 12:115-126, 1963.
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3. Feldman, H. and Rodbard, D. "Mathematical Theory of Radioimmunoassay", in: W.D. Odell and Doughaday, W.H. (Ed.), Principles of Competitive Protein-Binding Assays. Philadelphia: J.B. Leppincott Company; pp 158-203, 1971.
4. Westgard, J.O., et. al. A multi-rule Shewhart chart for quality control in clinical chemistry. *Clin. Chem.* 27:493-501, 1981.