MANUAL

HTRF Insulin Mouse Serum Detection Kit

Part # 62IN3PEF & 62IN3PEB

Test size#: 200 tests (62IN3PEF) and 5 x 200 tests (62IN3PEB) - assay volume: 20 µL

Revision: #06 of September 2023

Store at: 2-8°C (62IN3PEF); 2-8°C (62IN3PEB)

For research use only. Not for use in diagnostic procedures.

ASSAY PRINCIPLE

This kit is intended for the simple and rapid quantification of Insulin in mouse plasma and serum and offers a fast alternative to ELISA. The assay is not compatible with rat samples.

The detection principle of this kit is based on HTRF® technology (Homogeneous Time-Resolved Fluorescence). As shown in Figure 1, Insulin is detected in a sandwich assay by using anti-Insulin antibody labeled with Terbium cryptate (donor), and anti-Insulin antibody labeled with XL665 (acceptor).

When the dyes are in close proximity, the excitation of the donor with a light source (laser or flash lamp) triggers a Fluorescence Resonance Energy Transfer (FRET) towards the acceptor, which in turn fluoresces at a specific wavelength (665 nm). Signal intensity is proportional to the number of antigen-antibody complexes formed and therefore to the Insulin concentration.

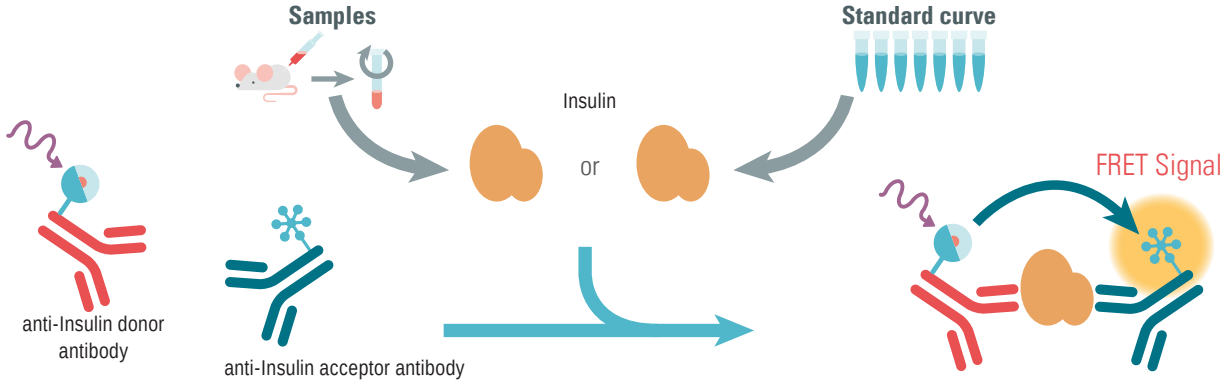
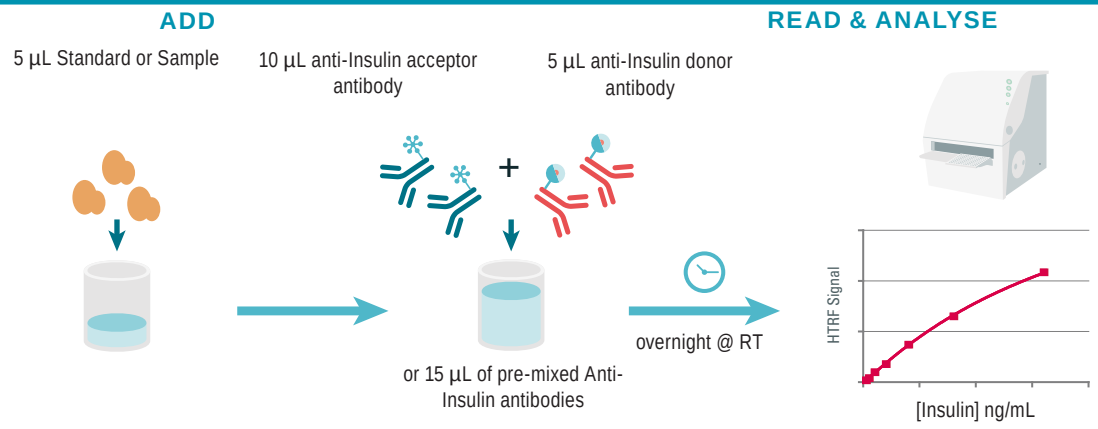


Figure 1: Principle of HTRF Insulin sandwich assay.

MANUAL AT A GLANCE



Make sure to use the set-up for Tb Cryptate.

MATERIALS PROVIDED:

KIT COMPONENTS	200 TESTS * CAT # 62IN3PEF	5 X 200 TESTS * CAT # 62IN3PEB
Insulin Standard Lyophilized	1 vial	5 vials
Insulin Tb Cryptate Antibody	1 vial Lyophilized	5 vials Lyophilized
Insulin XL665 Antibody	1 vial Lyophilized	5 vials Lyophilized
Diluent #6 ** ready-to-use	1 vial 6 mL	5 vials 6 mL
Detection buffer *** ready-to-use	1 vial 3 mL Detection Buffer #6	5 vials 3 mL Detection Buffer #6

* When used as advised, the two available kit sizes will provide sufficient reagents for 200 tests and 5 x 200 tests respectively in 20 µL final volume..
Assay volumes can be adjusted proportionally to run the assay in 96 or 1536 well microplates.
** Medium like cell culture medium can be an alternative to the diluent.
*** The Detection buffer is used to prepare working solutions of acceptor and donor reagents.

PURCHASE SEPARATELY:

- HTRF®-Certified Reader. Make sure the setup for Tb Cryptate is used.

For a list of HTRF-compatible readers and set-up recommendations, please visit www.revvity.com

- Small volume (SV) detection microplates - Use white plate only.

For more information about microplate recommendations, please visit our website at: www.revvity.com

STORAGE AND STABILITY

Store the kit at 2-8°C.

Under proper storage conditions, reagents are stable until the expiry date indicated on the label.

If lyophilized, reconstituted reagents, antibodies, and standard stock solutions may be frozen and thawed only once. To avoid freeze/thaw cycles, it is recommended to dispense remaining stock solutions into disposable plastic vials for storage at -60°C or below .

Volume of Insulin Mouse Serum standard aliquots should not be under 20 µL.

After first opening, store the diluent at -16°C or below.

REAGENT PREPARATION

BEFORE YOU BEGIN:

- It is very important to prepare reagents in the specified buffers. The use of an incorrect diluent may affect reagent stability and assay results.
- Allow the lyophilized reagents to warm up to room temperature for at least 30 mins before reconstitution
- Before use, allow Diluent and Detection buffer to warm up at room temperature and homogenize them with a vortex.
- It is recommended to filter buffers.
- Antibody solutions must be prepared in individual vials and can be mixed prior to dispensing.
- Insulin standards (for standard curve) must be prepared in diluent or in the same medium as the samples.

TAKE CARE TO PREPARE STOCK AND WORKING SOLUTIONS ACCORDING TO THE DIRECTIONS FOR THE KIT SIZE YOU HAVE PURCHASED.

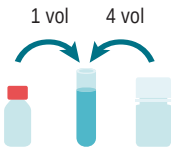
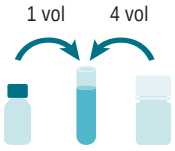
TO PREPARE REAGENT STOCK SOLUTIONS:

200 TESTS KIT - 62IN3PEF		5 X 200 TESTS KIT - 62IN3PEB
Anti-Insulin Tb Cryptate antibody		
Reconstitute the Insulin Tb Cryptate antibody with 200 µL distilled water. Mix gently. This 5 X stock solution can be frozen and stored at -60°C or below.		Reconstitute the Insulin Tb Cryptate antibody with 200 µL distilled water. Mix gently. This 5 X stock solution can be frozen and stored at -60°C or below.
Anti-Insulin XL665 antibody		
Reconstitute the Insulin XL665 antibody with 400 µL distilled water. Mix gently. This 5 X stock solution can be frozen and stored at -60°C or below.		Reconstitute the Insulin XL665 antibody with 400 µL distilled water. Mix gently. This 5 X stock solution can be frozen and stored at -60°C or below.
Insulin Standard		
Reconstitute the Insulin Standard with distilled water in order to obtain a 500 ng/mL stock solution. See instructions on vial label for reconstitution volume. Mix gently after reconstitution. This stock solution can be frozen and stored at -60°C or below.		Reconstitute the Insulin Standard with distilled water in order to obtain a 500 ng/mL stock solution. See instructions on vial label for reconstitution volume. Mix gently after reconstitution. This stock solution can be frozen and stored at -60°C or below.
Diluent		
The diluent is ready-to-use.		The diluent is ready-to-use.
Detection buffer		
The Detection buffer is ready-to-use.		The Detection buffer is ready-to-use.

TO PREPARE ANTIBODY WORKING SOLUTIONS:

Each well requires 5 µL of Insulin-Tb Cryptate Antibody and 10 µL of Insulin-XL665 Antibody.

Prepare the two antibody solutions in separate vials.

200 TESTS KIT - 62IN3PEF		5 X 200 TESTS KIT - 62IN3PEB
Insulin Eu Cryptate antibody		
Dilute 5-fold the 5 X stock solution (reconstituted reagent) of Anti Insulin Tb Cryptate antibody with the Detection buffer #6: add 1 volume of Tb Cryptate antibody stock solution in 4 volumes of Detection buffer #6 (e.g., 200 µL of reconstituted Tb Cryptate antibody stock solution + 800 µL of Detection Buffer #6).		Dilute 5-fold the 5 X stock solution (reconstituted reagent) of Insulin Tb Cryptate antibody with the Detection buffer #6: add 1 volume of Tb Cryptate antibody stock solution in 4 volumes of Detection buffer #6 (e.g., 200 µL of reconstituted Tb Cryptate antibody stock solution + 800 µL of Detection Buffer #6).
Insulin XL665 antibody		
Dilute 5-fold the 5 X stock solution (reconstituted reagent) of Insulin XL665 antibody with the Detection buffer #6: add 1 volume of XL665 antibody stock solution in 4 volumes of Detection buffer #6 (e.g., 400 µL of reconstituted Anti Insulin XL665 + 1600 µL of Detection Buffer #6).		Dilute 5-fold the 5 X stock solution (reconstituted reagent) of Insulin XL665 antibody stock solution with the Detection buffer #6: add 1 volume of XL665 antibody stock solution in 4 volumes of Detection buffer #6 (e.g., 400 µL of reconstituted XL665 antibody stock solution + 1600 µL of Detection Buffer #6).
Antibody mix		
It is possible to pre-mix the two ready-to-use antibody solutions just prior to dispensing the reagents by adding 2 volumes of XL665 antibody solution to 1 volume of Cryptate antibody solution (e.g. 1 mL of XL665 antibody + 0.5 mL of Cryptate antibody).		It is possible to pre-mix the two ready-to-use antibody solutions just prior to dispensing the reagents by adding 2 volumes of XL665-antibody solution to 1 volume of Cryptate antibody solution (e.g. 10 mL of XL665-antibody + 5 mL of Cryptate antibody).

TO PREPARE STANDARD WORKING SOLUTIONS:

- Each well requires 5 µL of standard.
- Dilute the standard stock solution serially with diluent #6
- In order to check for a potential interference effect from your own assay buffer when using the assay for the first time, we highly recommend the parallel preparation of a standard curve in your own supplemented cell culture medium and in diluent #6 .
- In order to counteract any standard sticking, we recommend changing tips between each dilution.

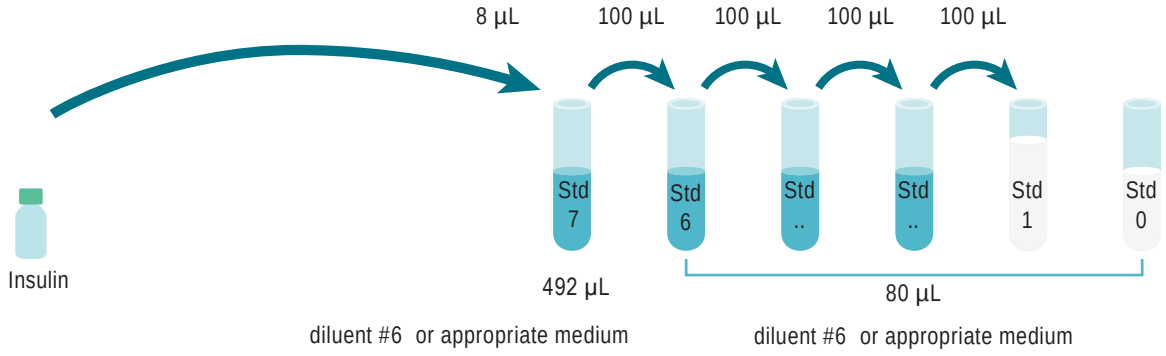
A recommended standard dilution procedure is listed and illustrated below:

Dilute the standard stock solution 62.5-fold with diluent #6 to prepare high standard (Std 7): e.g. take 8 µL of standard stock solution and add it to 492 µL of diluent #6 . Mix gently.

Use the high standard (Std 7) to prepare the standard curve using 1/1.8 serial dilutions as follows:

- Dispense 80 µL of diluent #6 in each vial from Std 6 to Std 0.
- Add 100 µL of standard to 80 µL of diluent #6 , mix gently and repeat the 1/1.8 serial dilution to make standard solutions: std6, std5, std4, std3, std2, std1.

This will create 7 standards for the analyte. Std 0 (Negative control) is diluent #6 or appropriate culture medium alone.



STANDARD	SERIAL DILUTIONS	INSULIN WORKING SOLUTIONS (ng/mL)
Standard Stock solution	Reconstituted lyophilisate	500
Standard 7	8 µL stock solution + 492 µL Diluent #6	8
Standard 6	100 µL standard 7 + 80 µL Diluent #6	4.44
Standard 5	100 µL standard 6 + 80 µL Diluent #6	2.47
Standard 4	100 µL standard 5 + 80 µL Diluent #6	1.37
Standard 3	100 µL standard 4 + 80 µL Diluent #6	0.76
Standard 2	100 µL standard 3 + 80 µL Diluent #6	0.42
Standard 1	100 µL standard 2 + 80 µL Diluent #6	0.24
Standard 0	80 µL Diluent #6	0

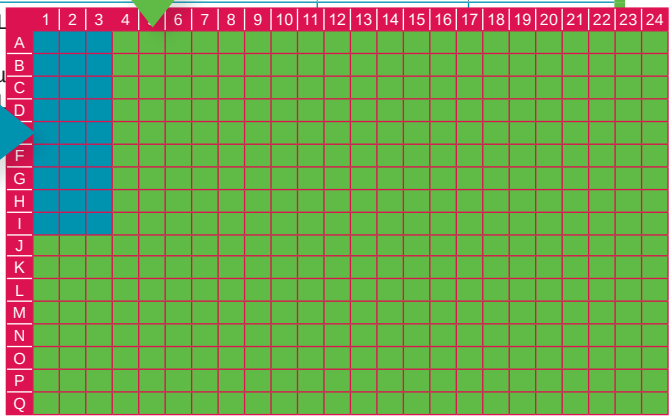
TO PREPARE SAMPLES:

- Each well requires 5 µL of sample.
- Just after their collection, put the samples at 4°C and test them immediately. For later use, samples should be dispensed into disposable plastic vials and stored at -60°C or below. Avoid multiple freeze/thaw cycles.
- In order to prepare plasma samples, we recommend collecting blood in EDTA-tubes.
- Samples with a concentration above the highest standard (Std 7) must be diluted diluent #6
- Avoid the use of samples with a high degree of hemolysis
- As Terbium Cryptate is sensitive to phenol red, it is mandatory to use medium without pheneol red (e.g. KRB or HSBC) to run your secretion assay

ASSAY MANUAL

	Standard (Std 0 - Std 7)	Samples
Step 1 	Dispense 5 µL of each Insulin standard (Std 0 - Std 7) into each standard well	Dispense 5 µL of each sample into each sample well
Step 2 	Add 10 µL of Insulin XL665 antibody working solution to all wells	
Step 3 	Add 5 µL of Insulin Tb Cryptate antibody working solution to all wells	
Step 4 	Seal the plate and incubate overnight @ RT	
Step 5	Remove the plate sealer and read on an HTRF® compatible reader	

	1	2	3	4	5	6
A	5 µL Std 0 (Negative control) 10 µL Insulin-XL665 5 µL Insulin-Tb Cryptate	Repeat Well A1	Repeat Well A1	5 µL Sample 1 10 µL Insulin-XL665 5 µL Insulin-Tb Cryptate	Repeat Well A4	Repeat Well A4
B	5 µL Std 1 10 µL Insulin-XL665 5 µL Insulin-Tb Cryptate	Repeat Well B1	Repeat Well B1	5 µL Sample 2 10 µL Insulin-XL665 5 µL Insulin-Tb Cryptate	Repeat Well B4	Repeat Well B4
C	5 µL Std 2 10 µL Insulin-XL665 5 µL Insulin-Tb Cryptate	Repeat Well C1	Repeat Well C1	5 µL Sample 3 10 µL Insulin-XL665 5 µL Insulin-Tb Cryptate	Repeat Well C4	Repeat Well C4
D	5 µL Std ... 10 µL Insulin-XL665 5 µL Insulin-Tb Cryptate	Repeat Well D1	Repeat Well D1	5 µL Sample ... 10 µL Insulin-XL665 5 µL Insulin-Tb Cryptate	Repeat Well D4	Repeat Well D4
E	5 µLStd ... 10 µL Insulin-XL665 5 µL Insulin-Tb Cryptate	Repeat Well E1	Repeat Well E1	5 µL Sample ... 10 µL Insulin-XL665 5 µL Insulin-Tb Cryptate	Repeat Well E4	Repeat Well E4
F	5 µL Std ... 10 µL Insulin-XL665 5 µL Insulin-Tb Cryptate	Repeat Well F1	Repeat Well F1	5 µL Sample ... 10 µL Insulin-XL665 5 µL Insulin-Tb Cryptate	Repeat Well F4	Repeat Well F4
G	5 µL Std ... 10 µL Insulin-XL665 5 µL Insulin-Tb Cryptate	Repeat Well G1	Repeat Well G1	5 µL Sample ... 10 µL Insulin-X 5 µL Insulin-Tb Cr	Repeat Well G4	Repeat Well G4
H	5 µL Std ... 10 µL Insulin-XL665 5 µL Insulin-Tb Cryptate	Repeat Well H1	Repeat Well H1	5 µL 10 µ		



DATA REDUCTION

1. Calculate the ratio of the acceptor and donor emission signals for each individual well.

$$\text{Ratio} = \frac{\text{Signal 665 nm}}{\text{Signal 620 nm}} \times 10^4$$

2. Calculate the % CVs. The mean and standard deviation can then be worked out from ratio replicates.

$$\text{CV (\%)} = \frac{\text{Standard deviation}}{\text{Mean Ratio}} \times 100$$

3. Calculate the % delta F which reflects the signal to background of the assay. The negative control (Standard 0) plays the role of an internal assay control. Delta F is used for the comparison of day to day runs of the same assay.

$$\text{delta F (\%)} = \frac{\text{Ratio Standard or sample - Ratio Negative Control}}{\text{Ratio Negative Control}} \times 100$$

For more information about data reduction, please visit www.revvity.com

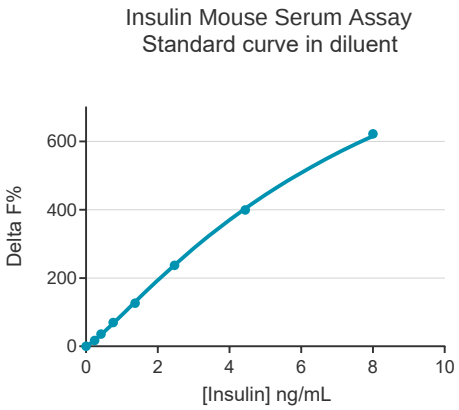
RESULTS

This data must not be substituted for the data obtained in the laboratory and should be considered only as an example.

Results may vary from one HTRF® compatible reader to another.

Standard curve fitting with the 4 Parameter Logistic (4PL) model:

	Ratio (1)	CV (2)	Delta F% (3)
Standard 0 - Negative control	1,055	2.3%	-
Standard 1 - 0.24 ng/mL	1,237	2.5%	17%
Standard 2 - 0.42 ng/mL	1,430	1.8%	36%
Standard 3 - 0.76 ng/mL	1,794	3.4%	70%
Standard 4 - 1.37 ng/mL	2,384	1.7%	126%
Standard 5 - 2.47 ng/mL	3,550	1.3%	237%
Standard 6 - 4.44 ng/mL	5,267	7%	399%
Standard 7 - 8 ng/mL	7,612	4.9%	622%



ANALYTICAL CHARACTERISTICS

ASSAY PERFORMANCES AND CALIBRATION

Calibration	NIBSC International standard 66/304 1 ng Insulin HTRF ⟷ 1 ng Insulin NIBSC 66/304 (i.e.: 2.3 x 10-05 IU)
Assay range	0.24 to 8 ng/mL
Limit of detection (LoD*) = Std 0 Mean + 2 SD	0.064 ng/mL
incubation time	Overnight at RT

*The LoD was calculated from data obtained with the PHERAstar Plus reader (flash lamp excitation) after overnight incubation. This may vary from one HTRFcompatible reader to another.

DILUTION LINEARITY

To assess the linearity of the assay, mouse samples with high concentration of Insulin were serially diluted with diluent # 6 to produce samples within the dynamic range of the assay. The insulin concentration measured was compared to the expected concentration.

Mouse serum sample	Dilution factor	[Insulin] expected (ng/mL)	[Insulin] measured (ng/mL)	% Recovery
1	1	-	0.97	-
	2.5	0.388	0.368	95%
	5	0.194	0.188	97%
2	1	-	4.00	-
	2.5	1.60	1.57	98%
	5	0.80	0.90	110%
3	1	-	7.10	-
	2.5	2.84	3.00	105%
	5	1.42	1.66	117%
Mean recovery after dilution				103.7%

SPIKE AND RECOVERY

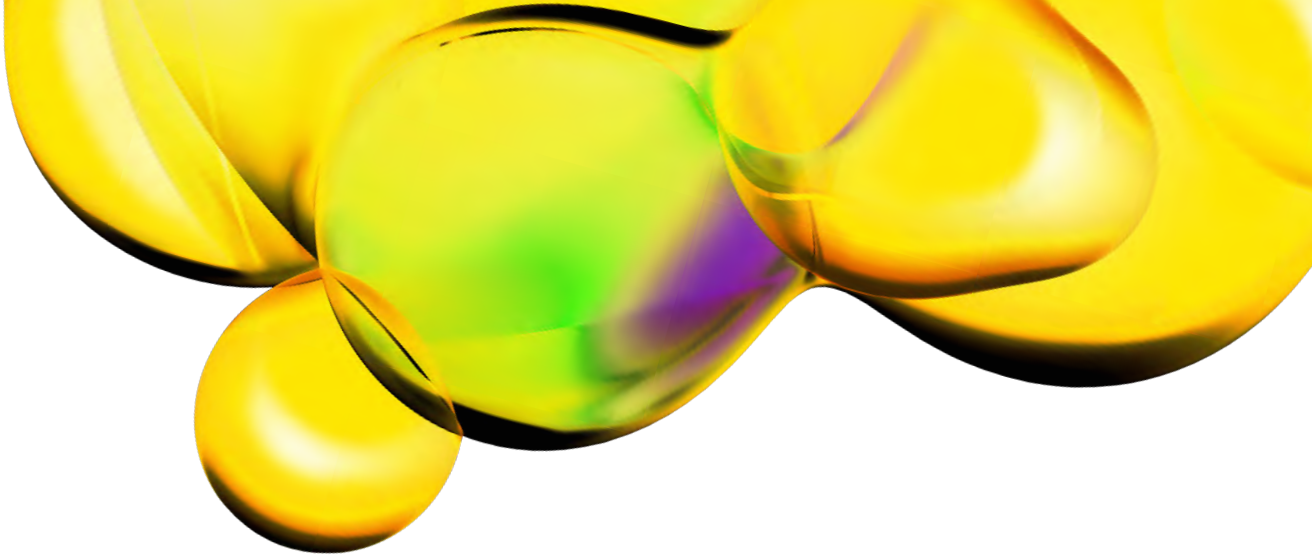
Mouse serum sample	[Insulin] added (ng/mL)	[Insulin] expected (ng/mL)	[Insulin] measured (ng/mL)	% Recovery
1	0	-	0.75	-
	2.3	3.05	3.68	118%
	4.8	5.55	6.4	115%
2	0	-	2.5	-
	2.4	4.9	4.8	98%
	4.8	7.3	8.0	110%
3	0	-	4.2	-
	0.8	5.0	5.4	108%
	1.6	5.8	6.2	107%
Mean recovery after spike				109.3%

INTRA-ASSAY VARIABILITY (N=24):

Mouse serum sample	Mean [Insulin] measured (ng/mL)	CV(%)
1	0.25	7.6%
2	0.73	3.7%
3	0.9	5.4%
4	2.84	4.6%
5	5.17	4.0%
Mean CV Intra-assay		5.1%

INTER-ASSAY VARIABILITY (N=4):

Mouse serum sample	Mean [Insulin] measured ng/mL	CV (%)
1	0.37	14.6%
2	0.67	9.5%
3	0.90	4.0%
4	1.10	8.1%
5	6.90	9.2%
Mean CV Inter-assay		9.1%



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