Lispro Insulin

250 Tubes

Cat. # LPI-16K

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LISPRO INSULIN RIA KIT
250 TUBES (Cat. # LPI-16K)

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LISPRO INSULIN RIA KIT
250 TUBES (Cat. # LPI-16K)

I. INTENDED USE

Lispro Insulin (Humalog®) is a synthetic human insulin analog in which the amino acid sequence of the B-chain at positions 28 (proline) and 29 (lysine) is inverted. The inversion significantly reduces molecular aggregations. Consequently, Lispro Insulin has a higher rate of absorption, higher peak serum levels and a shorter duration of action than regular human insulin following subcutaneous injection. This procedure describes a competitive-binding radioimmunoassay (RIA) for the quantitation of Lispro Insulin in serum, plasma or other biological fluids. The assay is highly specific for Lispro Insulin and has negligible level of cross-reactivity with native human insulin and proinsulin. ***For Research Use Only. Not for Use in Diagnostic Procedures.***

II. PRINCIPLES OF PROCEDURE

In this RIA, Lispro Insulin from either standards or unknown samples compete with ^{125}I-Lispro Insulin for binding sites on guinea pig antibody specific to Lispro Insulin during incubation. The antibody-antigen complex is then precipitated with a second antibody against guinea pig IgG in the presence of polyethylene glycol (PEG) and normal guinea pig serum as carrier. After centrifugation, the resulting pellets are counted for radioactivity in a gamma counter. Quantitation of Lispro Insulin in the unknown samples is achieved by interpolation from the standard curve.

Insulin therapy can result in the generation of anti-insulin antibodies in the treated patient or animal. These antibodies to insulin will cause analytical interference in this assay. An inexpensive and easy means to remove insulin antibodies from unknown samples has been described by Arnqvist et al, (Clin. Chem. 33:93-96, 1987) and is outlined in section VIII, page 10.

III. REAGENTS SUPPLIED

Each kit is sufficient to run 250 tubes and contains the following reagents:

A. Assay Buffer
0.05M Phosphosaline pH 7.4 containing 0.025M EDTA, 0.08% Sodium Azide, and 1% RIA Grade BSA
Quantity: 40 mL/vial
Preparation: Ready to use

B. Lispro Insulin Antibody
Guinea Pig anti-Lispro Insulin Serum in Assay Buffer
Quantity: 26 mL/vial
Preparation: Ready to use

C. ^{125}I-Lispro Insulin
^{125}I-Lispro Insulin Label, HPLC purified (specific activity 367 µCi/µg)
Lyophilized for stability. Freshly iodinated label contains <3 µCi (111 kBq) calibrated to the 1st Monday of each month.
Quantity: 27 mL/vial upon hydration
Preparation: Contents Lyophilized. Hydrate with entire contents of Label Hydrating Buffer. Allow to sit at room temperature for 30 minutes, with occasional gentle mixing.

D. Label Hydrating Buffer
Assay Buffer containing Normal Guinea Pig IgG as a carrier. Used to hydrate ^{125}I-Lispro Insulin.
Quantity: 27 mL/vial
Preparation: Ready to use

E. Lispro Insulin Standard
Purified Lispro Insulin in Assay Buffer at the following concentration:
250 µU/mL
Quantity: 3 mL/vial
Preparation: Must be serial diluted.

F. Matrix Solution
For correction of matrix effect in serum and plasma samples. Solution contains 0.08% Sodium Azide
Quantity: 6.5 mL/vial
Preparation: Ready for use.

G. Quality Controls 1 & 2
Lispro Insulin in QC buffer.
Quantity: 2 mL/vial
Preparation: Ready to use.

H. 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, pH 7.4.
Quantity: 260 mL/vial
Preparation: Ready to use; chill to 4°C.

IV. STORAGE AND STABILITY

Upon receipt, unused kit may be stored between 2 and 8°C for short term storage. For prolonged storage (>2 weeks), freeze unused kit at ≤ -20°C. Lyophilized components upon hydration should be stored at ≤-20°C immediately after use, or discarded. Avoid multiple (>5) 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 and are unopened.

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.

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 easy disposal of small amounts of contamination.
9. Wipe up all spills immediately and thoroughly and dispose of the contaminated materials as radioactive waste. Inform Radiation Safety Officer.

V. REAGENT PRECAUTIONS (continued)

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. Dispose of unused contents and waste in accordance with international, federal, state, and local regulations.

Full labels of hazardous components in this kit:

Ingredient, Cat #		Full Label	
^{125} I-LisPro Insulin Tracer	9016		**Danger.** Harmful if swallowed. Causes serious eye damage. Harmful to aquatic life with long lasting effects. Avoid release to the environment. Wear eye protection. IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. Get medical advice/ attention.
LisPro Insulin Antibody	1016-K		**Warning.** Causes serious eye irritation. IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
Precipitating Reagent	PR-UV		**Warning.** Causes serious eye irritation. IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

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 µL pipet with disposable tips
3. 100 µL & 1.0 mL repeating dispenser
4. Refrigerated swing bucket centrifuge capable of developing 2,000 - 3,000 x g. (Fixed-angle buckets are not recommended.)
5. Absorbent paper
6. Vortex mixer
7. Refrigerator
8. Gamma Counter
9. Polyethylene glycol, if removal of insulin antibody from samples is desired.

VII. SPECIMEN COLLECTION AND STORAGE

1. To prepare serum, whole blood is directly collected into a Vacutainer® serum tube that contains no anticoagulant. Let blood clot at room temperature for 30 minutes.

2. Promptly centrifuge the clotted blood at 2000 to 3000 x g for 15 minutes at 4°C.

3. Transfer and store the serum in separate tubes. Date and identify each sample.

4. Store serum samples in lab freezer at –20°C.

5. To prepare plasma, blood should be collected into Vacutainer® EDTA-plasma tubes and centrifuged immediately after collection.

6. Care must be taken when using heparin as an anticoagulant, since an excess will cause falsely high values. (Thorell and Lanner, 1973). Use no more than 10 IU heparin per mL of blood collected.

VIII. ASSAY PROCEDURE

Helpful Notes:

1. Before attempting the assay, read and familiarize yourself with assay procedures and select the appropriate assay option.
2. Samples are typically assayed in duplicate, although other multiples may be used to obtain a more accurate average value.
3. For optimal results, accurate pipetting of all reagents and strict adherence to the protocol are recommended.

Assay Options:

Assay Option #1: Serum and plasma without endogenous insulin antibodies can be assayed directly by the following procedures without modification of samples. Insulin antibodies are often formed during therapeutic treatment with insulin. Refer to **Flow Chart #1** (Lispro Insulin Assay for Unmodified Samples) for a suggested arrangement of samples and sample volume to be assayed. When assaying a large number of samples, additional quality controls may be included near the middle and at the end of the assay.

Standard Preparation

Use care in opening the Standard vial.

Label six glass tubes 1, 2, 3, 4, 5, and 6. Add 1.0 mL Assay Buffer to each of the six tubes. Prepare serial dilutions by adding 1.0 mL of the 250uU/mL standard to tube 1, mix well and transfer 1.0 mL of tube 1 to tube 2, mix well and transfer 1.0 mL of tube 2 to tube 3, mix well and transfer 1.0 mL of tube 3 to tube 4, mix well and transfer 1.0 mL of tube 4 to tube 5, mix well and transfer 1.0 mL of tube 5 to tube 6, mix well.

Note: Do not use a Repeater pipette. Change tip for every dilution. Wet tip with Standard before dispensing. Unused portions of standard should be stored at ≤ -20°C. Avoid multiple freeze/thaw cycles.

Tube #	Standard Concentration	Volume of Assay Buffer to Add	Volume of Standard to Add
1	125 uU/mL	1.0 mL	1.0 mL of 250 uU/mL
2	62.5 uU/mL	1.0 mL	1.0 mL of 125 uU/mL
3	31.25 uU/mL	1.0 mL	1.0 mL of 62.5 uU/mL
4	15.63 uU/mL	1.0 mL	1.0 mL of 31.25 uU/mL
5	7.813 uU/mL	1.0 mL	1.0 mL of 15.63 uU/mL
6	3.906 uU/mL	1.0 mL	1.0 mL of 7.813 uU/mL

VIII. ASSAY PROCEDURE (continued)

Day One

1. Pipette 300 µL Assay Buffer into the Non-Specific Binding (NSB) tubes (#3, 4) and 100 µL Assay Buffer into the Reference tubes (#5, 6) and sample tubes (#25 through the end).

2. Pipette sequentially 100 µL Standards and Quality Controls in duplicate into tubes #7 to 24.

3. Pipette sequentially 100 µL samples in duplicate into tubes #25 to the end. If sample volume is less than 100 µL, see notes in the Flow Chart.

4. Pipette sequentially 100 µL Matrix Solution-PS into tubes #5 to 24. See notes in the Flow Chart.

5. Pipette 100 µL hydrated Lispro Insulin label into all tubes.

6. Pipette 100 µL Lispro Insulin antibody into all tubes except Total Count tubes (#1, 2) and NSB tubes (#3, 4).

7. Vortex, cover tubes and incubate for 20-24 hours at room temperature.

Day Two

8. Add 1.0 mL Precipitating Reagent to all tubes except Total Count tubes (#1, 2).

9. Vortex and incubate 20 minutes at 4°C.

10. Centrifuge tubes at 3000 x g at 4°C for 25 minutes.

11. Immediately decant supernatant and drain the tubes for 20 to 30 seconds.

12. Count pellets in a gamma counter following the manufacturer's instructions.

13. Calculate results according to Section IX.

VIII. ASSAY PROCEDURE (continued)

Assay Option #2: When a sample of serum or plasma contains insulin antibodies, the latter have to be removed from the sample prior to the assay. The antibodies can be extracted with polyethylene glycol (PEG) according the following procedures. However, the introduction of PEG to the sample necessitates similar treatment of calibration standards and quality controls with PEG.

Extraction of Calibration Standards, QCs, and Serum or Plasma Samples by PEG

Refer to **Flow Chart #2** for a suggested extraction protocol to generate enough material for an assay of duplicate sampling tubes. For assays of samplings higher than duplicates, increase all components proportionally for the extraction. **The PEG is not provided in this kit, but can be purchased from other sources.** Borosilicate tubes of 12 x 75 mm size can be used as extraction tubes.

1. Dissolve 25 grams of PEG [molecular weight 7000~9000] with 0.9% NaCl solution to a final volume of 100 mL. The 25% PEG is stored at 4 °C and should be used within 2 weeks after preparation.

2. Add 300 µL Assay Buffer to extraction tube #1 and 175 µL to extraction tubes #10 to the end.

3. Add 175 µL of Lispro Insulin Standards, from 7.813 µU/mL to 250 µU/mL, to extraction tubes #2 to 7, respectively.

4. Add 175 µL QC Low and QC High to extraction tube #8 and 9, respectively.

5. Add 175 µL serum or plasma samples to extraction tubes #10 to the end.

6. Add 300 µL Matrix Solution-PS to extraction tube #1 and 175 µl to extraction tubes #2 to 9.

7. Add 600 µL 25% PEG solution to extraction tube #1 and 350 µL to extraction tubes #2 to the end.

8. Vortex and incubate all extraction tubes at 4 °C for 25 minutes.

9. Centrifuge all extraction tubes in a swinging bucket rotor at 4°C with 2000 to 3000 xg for 25 minutes.

10. Carefully transfer the supernatants to another set of clean empty tubes and discard the pellets. Store supernatants at 4°C until the time of assay.

VIII. ASSAY PROCEDURE (continued)

Assay Lispro Insulin content in the supernatants according to the following protocol. Refer to **Flow Chart #3** (Lispro Insulin Assay for PEG Extracted Samples) for a suggested arrangement of samples. When assaying a large number of samples, additional Quality Controls may be included near the middle and at the end of the assay. To accommodate higher amount of QCs required, extract proportionally more QCs with PEG prior to the assay.

Day One

1. Pipette 100 µL Assay Buffer into the Non-Specific Binding (NSB) assay tubes (#3, 4).
2. Pipette 200 µL supernatant from extraction tube #1 into NSB assay tubes (#3, 4) and into the reference (Bo) assay tubes (#5, 6).
3. Pipette 200 µL supernatants in duplicates from extraction tubes #2-9 (PEG extracted Lispro Insulin Standards and QCs) into assay tubes #7-22.
4. Pipette 200 µL supernatants in duplicates from extraction tubes #10 and up (PEG extracted samples) into assay tubes #23 to the end. If sample supernatant needs to be diluted, see notes in the flow chart.
5. Pipette 100 µL hydrated Lispro Insulin label into all assay tubes.
6. Pipette 100 µL Lispro Insulin antibody into assay tubes #5 to the end.
7. Vortex, cover tubes and incubate for 20-24 hours at room temperature.

Day Two

8. Add 1.0 mL Precipitating Reagent to all tubes except Total Count tubes (#1, 2)
9. Vortex and incubate 20 minutes at 4°C.
10. Centrifuge tubes at 3000 x g at 4°C for 25 minutes.
11. Immediately decant supernatant and drain the tubes for 20 to 30 seconds.
12. Count pellets in a gamma counter following the manufacturer's instructions.
13. Calculate results according to Section IX.

VIII. ASSAY PROCEDURE (continued)

Flow Chart #1: Lispro Insulin Assay for Unmodified Samples

[**Note:** This method is optimized for quantification of Lispro Insulin in unmodified human serum or plasma. To measure Lispro Insulin in serum or plasma samples other than human source, it may be necessary to adjust the amount of Matrix Solution-PS used in the assay. In addition, all sample volumes within an assay should be kept constant. If sample volume is less than 100 µL, choose one of the following alternatives in setting up the assay: 1) make up the deficiency in sample volume from 100 µl with pooled human serum or plasma from healthy non-diabetic subjects with no recent history of Lispro Insulin injection; or 2) use Matrix Solution-PS in tubes 5-24 in a quantity identical to the sample volume used in tubes 25-n and make up the volume deficiency from 100 µL in both with Assay Buffer. The first alternative has to be selected if different sample volumes are to be used among the unknowns within an assay. In both cases, the results obtained should be corrected for the appropriate dilution factor in the assay.]

First Day							Second Day		
	Step 1	Step 2-3	Step 4	Step 5	Step 6	Step	Step 8	Step 9	Step
Tube #	**Assay Buffer**	**Standard/QC/Sample**	**Matrix Solution-PS**	**^{125}I-Lispro Insulin**	**Lispro Insulin Antibody**	**Vortex, Cover, and Incubate 20-24 hrs at RT**	**Add Precipitating Reagent**	**Vortex, and Incubate 20 min. at 4°C**	**Centrifuge at 4°C for 25 min., Decant, and Count pellets**
1,2	----	----	----	100 µL	----		-		
3,4	300 µL	----	----	100 µL	----		1.0 mL		
5,6	100 µL	----	100 µL	100 µL	100 µL		1.0 mL		
7,8	----	100 µL of 3.906 µU/	100 µL	100 µL	100 µL		1.0 mL		
9,10	----	100 µL of 7.813 µU/	100 µL	100 µL	100 µL		1.0 mL		
11,12	----	100 µL of 15.63 µU/	100 µL	100 µL	100 µL		1.0 mL		
13,14	----	100 µL of 31.25 µU/	100 µL	100 µL	100 µL		1.0 mL		
15,16	----	100 µL of 62.5 µU/ mL	100 µL	100 µL	100 µL		1.0 mL		
17,18	----	100 µL of 125 µU/ mL	100 µL	100 µL	100 µL		1.0 mL		
19,20	----	100 µL of 250 µU/ mL	100 µL	100 µL	100 µL		1.0 mL		
21,22	----	100 µL of QC, Low	100 µL	100 µL	100 µL		1.0 mL		
23,24	----	100 µL of QC, High	100 µL	100 µL	100 µL		1.0 mL		
25,26	100 µL	100 µL of Sample # 1	----	100 µL	100 µL		1.0 mL		
27-n	100 µL	100 µL of Sample # 2 –	----	100 µL	100 µL		1.0 mL		

VIII. ASSAY PROCEDURE (continued)

Flow Chart #2: Polyethylene Glycol Extraction of Standards, QCs, and Serum or Plasma Samples

1. Mix the following components in 12 x 75 mm borosilicate tubes according to the chart below: [Note: Because of the high viscosity of PEG solution, precision in pipetting the 25% PEG solution and PEG extracted samples is crucial to the success of this assay]

Extraction Tube #	Assay Buffer	Standards, QCs, Samples	Matrix Solution-PS	25% PEG in 0.9 % NaCl
1	300 µL	----	300 µL	600 µL
2	----	175 µL of 7.813 µU/mL	175 µL	350 µL
3	----	175 µL of 15.63 µU/mL	175 µL	350 µL
4	----	175 µL of 31.25 µU/mL	175 µL	350 µL
5	----	175 µL of 62.5 µU/mL	175 µL	350 µL
6	----	175 µL of 125 µU/mL	175 µL	350 µL
7	----	175 µL of 250 µU/mL	175 µL	350 µL
8	----	175 µL of QC, Low	175 µL	350 µL
9	----	175 µL of QC, High	175 µL	350 µL
10	175 µL	175 µL of Sample # 1	----	350 µL
11-n	175 µL	175 µL of Sample # 2 - end	----	350 µL

2. Mix well by vortex. Incubate at 4°C for 25 minutes. Centrifuge in a swinging bucket rotor at 4°C and 2000 to 3000 x g for 25 minutes.

3. Carefully transfer the supernatants to clean empty tubes or vials with ID numbers and discard pellets. Assay supernatants per assay instructions.

VIII. ASSAY PROCEDURE (continued)

Flow Chart #3: Lispro Insulin Assay for PEG Extracted Samples

[**Note:** This method is optimized for quantification of Lispro Insulin in human serum or plasma after PEG extraction. To measure Lispro Insulin in serum or plasma samples other than human source, it may be necessary to adjust the amount of Matrix Solution-PS used in the assay. If PEG extracted samples need to be diluted, use a diluent prepared as follows. To one volume of pooled serum or plasma from healthy non-diabetic subjects, add one volume of Assay Buffer and two volumes of 25% PEG dissolved in 0.9% NaCl. Mix well and incubate at 4°C for 20 min. Centrifuge the mixture at 4°C and 3000 x g for 20 min. Collect the supernatant and use as sample diluent.]

First Day						Second Day		
	Step 1	**Step 2-4**	**Step 5**	**Step 6**	**Step 7**	**Step 8**	**Step 9**	**Step 10-12**
Tube #	**Assay Buffer**	**Supernatant After PEG Extraction (Supernatant from extraction tubes)**	**^{125}I-Lispro Insulin**	**Lispro Insulin Antibody**	**Vortex, Cover, and Incubate 20-24 hrs at RT**	**Add Precipitating Reagent**	**Vortex, and Incubate 20 min. at 4°C**	**Centrifuge at 4°C for 25 min., Decant, and Count pellets**
1,2	----	----	100 µL	----		-		
3,4	100 µL	200 µL Tube # 1 (of Matrix Solution-PS)	100 µL	----		1.0 mL		
5,6	----	200 µL Tube # 1 (of Matrix Solution-PS)	100 µL	100 µL		1.0 mL		
7,8	----	200 µL Tube # 2 (of 7.813 µU/mL Standard)	100 µL	100 µL		1.0 mL		
9,10	----	200 µL Tube # 3 (of 15.63 µU/mL Standard)	100 µL	100 µL		1.0 mL		
11,12	----	200 µL Tube # 4 (of 31.25 µU/mL Standard)	100 µL	100 µL		1.0 mL		
13,14	----	200 µL Tube # 5 (of 62.5 µU/mL Standard)	100 µL	100 µL		1.0 mL		
15,16	----	200 µL Tube # 6 (of 125 µU/mL Standard)	100 µL	100 µL		1.0 mL		
17,18	----	200 µL Tube # 7 (of 250 µU/mL Standard)	100 µL	100 µL		1.0 mL		
19,20	----	200 µL Tube # 8 (of QC, Low)	100 µL	100 µL		1.0 mL		
21,22	----	200 µL Tube # 9 (of QC, High)	100 µL	100 µL		1.0 mL		
23,24	----	200 µL Tube # 10 (of Sample #1)	100 µL	100 µL		1.0 mL		
25-n	----	200 µL Tube # 11-n (of Sample #2 – end)	100 µL	100 µL		1.0 mL		

IX. CALCULATIONS AND TRANSFORMATIONS

A. Automated Calculations:

1. A gamma counter with a data reduction system can automatically perform the calculations for Lispro-Insulin assay. Several data transformation programs are provided by the manufacturer of the gamma counter (e.g., four-parameter, weighted log/logit, spline, etc.). For this assay, four-parameter data reduction is the method of choice.

2. Results are reported in μU/mL in unknown sample. When sample volumes assayed differ from 100 µL, an appropriate mathematical adjustment must be made to accommodate for the dilution factor (e.g., if 50 µL of sample is used, then the calculated data must be multiplied by 2).

B. Manual Calculations:

1. Average duplicate counts for Total Counts, NSB, Total Binding (Bo or reference) tubes and remaining standards and samples.

2. Subtract the average NSB counts from each average count except the Total counts. These are the counts used in the following calculations.

3. Calculate the percentage of tracer (Label) bound. [%Bo = (Bo count/total count) x 100].

4. Calculate the percentage of maximum binding (%B/Bo) for each standard and sample.

   [%B/Bo = (sample of standard/total binding) x 100].

5. Plot the changes in%B/Bo as a function of concentrations of Lispro Insulin standards on a log-log graph paper.

6. Construct the standard curve by joining the points with a smooth curve.

7. Determine the concentrations of the Lispro Insulin in quality controls and unknown samples by interpolation of the standard curve.

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 Lispro Insulin assay is 3.906 µU/mL (100 µL sample size of unmodified samples), and 7.813 µU/mL (200 µL sample size of PEG extracted samples).

4. The limit of linearity for the Lispro Insulin assay is 250 µU/mL (100 µL sample size). Any result greater than 250 µU/mL should be repeated on dilution using assay buffer as a diluent.

XI. ASSAY CHARACTERISTICS

A. Sensitivity

The lowest level of Lispro Insulin that can be detected with Assay Option #1 is 3.9 µU/mL when using a 100 µL sample size, and 7.8 µU/mL with Assay Option #2 when using 200 µL of extracted sample.

B. Performance

The following parameters of assay performance are expressed as Mean ± Standard Deviation.

Assay Option #1: Unmodified Samples

ED_{80} = 10 ± 1 µU/mL
ED_{50} = 36 ± 2 µU/mL
ED_{20} = 136 ± 5 µU/mL

Assay Option #2: PEG Extracted Samples

ED_{80} = 16 ± 1 µU/mL
ED_{50} = 69 ± 3 µU/mL
ED_{20} = 289 ± 9 µU/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.

Human Proinsulin	<0.05%
Human Insulin	<0.05%

XII. QUALITY CONTROLS

Good Laboratory Practice (GLP) requires that Quality Control 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 EMD Millipore website emdmillipore.com using the catalog number as the keyword.

Recommended batch analysis decision using two controls (Westgard Rule)[3]:

1. When both controls are within ±2 SD. Decision: Approve batch and release analytical results.

2. When one control is outside ±2 SD and the second control is within ±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

XIII. REPLACEMENT REAGENTS

Reagents	Cat. #
^{125}I-Lispro Insulin (<3 µCi, 111 kBq)	9016
Label Hydrating Buffer (27mL)	LHB-P
Lispro Insulin Standards (2 mL each)	8016-K
Matrix Solution (6.5 mL/each)	PS0016
Lispro Insulin Antibody (26 mL)	1016-K
Precipitating Reagent (260 mL)	PR-UV
Quality Control 1 and 2 (2 mL each)	6016-K
Assay Buffer (40 mL)	AB-P

XIV. ORDERING INFORMATION

To place an order or to obtain additional information about our immunoassay products, please contact your Customer Service or Technical Support Specialist.

Contact information for each region can be found on our website:

emdmillipore.com/contact

Conditions of Sale

For Research Use Only. Not for Use in Diagnostic Procedures.

Safety Data Sheets (SDS)

Safety Data Sheets for EMD Millipore products may be ordered by fax or phone or through our website at emdmillipore.com/msds.

XV. REFERENCES

1. Arnquist H, Olsson P-O, and Von Schenck H. Free and total insulin as determined after precipitation with polyethylene glycol: analytical characteristics and effects of sample handling and storage. *Clin Chem* 33:93-96 (1987).

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3. Westgard, J.D. et. al. A multi-rule Shewhart chart for quality control in clinical chemistry. Clin. Chem. 27:493-501, 1981.