Version 22-04/24

User's Manual

Lispro NL-ELISA Kit

REF **DEIASL534**

Σ **96T**

RUO

This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

Creative Diagnostics

Address: 45-1 Ramsey Road, Shirley, NY 11967, USA

Tel: 1-631-624-4882 (USA) 44-161-818-6441 (Europe) **Fax: 1-631-938-8221**

Email: info@creative-diagnostics.com **Web: www.creative-diagnostics.com**

PRODUCT INFORMATION

Intended Use

The Lispro NL-ELISA provides a method for the quantitative determination of insulin lispro in plasma or serum samples.

General Description

The Lispro NL-ELISA is a ligand binding assay able to measure insulin lispro specifically, without cross-reaction to native insulin, native proinsulin, or any of the tested insulin analogues. Also, no interference from insulin autoantibodies (IgG antibodies), which can be present in samples from diabetic patients, has been observed. The Lispro NL-ELISA is designed to fit the needs of the pharmaceutical industry, by meeting the requirements specified in the EMA/FDA guidelines.

Principles of Testing

The Lispro NL-ELISA is a solid phase two-site enzyme immunoassay based on the sandwich technique, in which two monoclonal antibodies are directed against separate antigenic determinants on the lispro molecule. Insulin lispro in the sample reacts with anti-lispro antibodies bound to microtitration wells and peroxidase-conjugated anti-lispro antibodies in the solution. A simple washing step removes unbound enzyme-labelled antibody. The bound conjugate is detected by reaction with the chemiluminescent substrate. A chemiluminescence plate reader is used to read the intensity of light generated.

Reagents And Materials Provided

Each CD Lispro NL-ELISA kit reagents for 96 wells, sufficient for 36 samples, 3 controls and one calibrator curve in duplicate. For larger series of assays, use pooled reagents from packages bearing identical lot numbers. The expiry date for the complete kit is stated on the outer label. The recommended storage temperature is 2–8°C.

1. Coated Plate, 1 plate 96 wells, Ready for Use

Mouse monoclonal anti-lispro. For unused microplate wells completely reseal the bag using adhesive tape, store at 2–8°C and use within 4 weeks.

2. Calibrators 1, 2, 3, 4, 5, 6, 6 vials 500 µL. Lyophilized

Serum-based. Concentration stated on vial label. Reconstituted Calibrators are stable for 1 month at 2-8°C.

3. Calibrator 0, 1 vial 500 µL. Lyophilized

Serum-based. Reconstituted Calibrators are stable for 1 month at 2-8°C.

4. Anchor Points Low, High, 2 vials 500 µL. Lyophilized

Serum-based. Concentration stated on on vial label. Reconstituted Anchor Points are stable for 1 month at 2-8°C.

5. Controls Low, Medium, High, 3 vials 500 µL. Lyophilized

Serum-based. Concentration stated on on vial label. Reconstituted Controls are stable for 1 month at 2-8°C.

6. Assay Buffer, 1 vial 14 mL.

Color coded red

7. Enzyme Conjugate 11×, 1 vial 1.3 mL

Peroxidase conjugated mouse monoclonal anti-insulin

8. Enzyme Conjugate Buffer, 1 vial 13 mL

Color coded blue

9. Wash Buffer 21×, 1 bottle 50 mL

Storage after dilution: redistilled water

10. Substrate NL Ultra A, 1 vial 2 mL

Colorless solution

11. Substrate NL Ultra B, 1 vial 4 mL

Colorless solution

Materials Required But Not Supplied

1. Pipettes with appropriate volumes (multichannel or repeating pipettes preferred for addition of Assay Buffer, enzyme conjugate 1× solution and substrate reagent solution)
2. Tubes, beakers and cylinders for reagent preparation
3. Redistilled water for resuspension of calibrators and controls
4. Magnetic stirrer
5. Vortex mixer
6. Microplate reader for chemiluminescence (glow)
7. Microplate shaker (700–900 cycles per minute. orbital movement)
8. Microplate washing device with overflow function (recommended but not required)

Storage

Store between 2-8°C

Specimen Collection And Preparation

Serum, EDTA plasma and P-800 plasma can be used. Store samples at -80°C and avoid freeze-thaw cycles. Up to three freeze-thaw cycles for serum and plasma samples had minimal effect on lispro levels in samples. Avoid long time storage of samples at room temperature or 2-8°C. Lispro in serum and plasma samples was found to be stable at room temperature for 4 hours and at 2-8°C for 24 hours.

1. Serum

Collect blood by venipuncture, allow to clot and separate the serum by centrifugation. Store samples at -80°C and avoid freeze-thaw cycles. Avoid storing samples at room temperature or 2-8°C.

2. EDTA plasma and P-800 plasma

Collect blood by venipuncture into tubes containing EDTA as anticoagulant, and separate the plasma fraction by centrifugation. Store samples at -80°C and avoid freeze-thaw cycles. Avoid storing samples at room temperature or 2-8°C.

3. Preparation of samples

No dilution is normally required, however, samples above the obtained value of Calibrator 6 should be diluted with Calibrator 0 or a serum pool. Note! Buffers containing sodium azide (NaN_3) cannot be used for sample dilution.

Reagent Preparation

1. Calibrators 1, 2, 3, 4, 5, 6

Resuspend with 500 µL redistilled water per vial.

2. Calibrator 0

Resuspend with 500 µL redistilled water per vial.

3. Anchor Points Low, High

Resuspend with 500 µL redistilled water per vial.

4. Controls Low, Medium, High

Resuspend with 500 µL redistilled water per vial.

5. Wash Buffer

Dilute with 1000 mL redistilled water to make wash buffer 1× solution.

6. Preparation of enzyme conjugate 1× solution

Prepare the needed volume of Enzyme Conjugate 1× solution by dilution of Enzyme Conjugate 11×, (1+10) in Enzyme Conjugate Buffer according to the table below. When preparing enzyme conjugate 1× solution for the whole plate, pour all the blue Enzyme Conjugate Buffer into the Enzyme Conjugate 11× vial. Mix gently. Store at 2-8°C and use within 4 weeks.

Number of strips	Enzyme Conjugate 11X	Enzyme Conjugate Buffer
12 strips	1 vial	1 vial
8 strips	700 µL	7000 µL
4 strips	350 µL	3500 µL

7. Preparation of substrate working solution

Prepare the needed volume of substrate working solution by mixing Substrate NL Ultra A with Substrate NL Ultra B (1:2) according to the table below. Mix gently. Store at 2-8°C and use within 1 day. Note! Protect substrate from light.

Number of strips	Substrate NL Ultra A	Substrate NL Ultra B
12 strips	1 vial	1 vial
8 strips	1.2 mL	2.4 mL
4 strips	0.6 mL	1.2 mL

Assay Procedure

All reagents and samples must be brought to room temperature before use. Assay a calibrator curve in each run. Keep substrate and conjugate solution separated from each other during all steps to avoid contamination. The product has been optimized and validated without plate sealer.

1. Prepare enzyme conjugate 1× solution, substrate working solution and wash buffer 1× solution.
2. Resuspend Calibrators, Anchor Points and Controls with 500 µL redistilled water.
3. Prepare sufficient microplate wells to accommodate Calibrators, Anchor Points, Controls and samples in duplicate.
4. Pipette 10 µL each of Calibrators, Anchor Points, Controls and samples into appropriate wells.
5. Add 100 µL of Assay Buffer into each well.
6. Incubate on plate shaker for 1 hour (700-900 rpm) at room temperature (18-25°C).
7. On an automatic plate washer, use plate mode combined with overflow wash function to wash the plate with 700 µL wash buffer 1× solution per well for 6 cycles. Invert and tap the plate firmly against absorbent paper after the final wash. Or manually, discard the reaction volume by inverting the microplate over a sink. Add 350 µL wash buffer 1× solution to each well. Discard the wash buffer 1× solution, tap firmly several times against absorbent paper to remove excess liquid. Repeat 5 times for a total of 6 cycles.
8. Add 100 µL enzyme conjugate 1× solution to each well.
9. Incubate on plate shaker for 1 hour (700-900 rpm) at room temperature (18-25°C).
10. Wash as described in step 7.
11. Add 50 µL substrate working solution into each well.
12. Incubate for 10 minutes (18-25°C).
13. Read the luminosity of the wells using a 1 second integration time, without using a filter. If the reader has manual gain, gain should be set so that the signal of Anchor Point High is within the readers dynamic range. Read within 15 minutes.
14. Concentrations of Controls and samples should be read/calculated using Anchor Points and Calibrator 1-6 with a 5-pl weighted fit ($1/Y^2$). Note! Calibrator 0 should not be included in the curve fit.

Quality Control

Serum controls included in the kit and/or internal plasma/serum pools with low, intermediate and high insulin lispro concentrations should routinely be assayed as samples and results should be charted from day to day. It is good laboratory practice to record the following data for each assay: kit lot number, preparation dates of kit components, calibrators and controls.

Laboratories should follow government regulations or accreditation requirements for quality control frequency. The concentration ranges stated on the control vials are based on the nominal value of insulin lispro with

acceptance criteria 100% ± 20% as recommended by EMA/FDA.

Calculation

The concentration of lispro is obtained by plotting the relative light units (RLU) of the Calibrators, except for Calibrator 0, versus their concentration. It is important to use an appropriate curve fitting model that represents the true dose-response relationship to get accurate results. It is every laboratory's responsibility to try out the functionality of the chosen curve fitting model and used software. Note that weighting of the curve fit is important to get a proper fit at the low range of the standard curve, especially when the measuring range is wide.

The Lispro NL-ELISA is validated using MARS (BMG Labtech) with Five Parameter Logistic (5PL) and automatic weighting using $1/Y^2$.

Note!

1. Include the Anchor Points in the curve fit.
2. Do not include Calibrator 0 in the curve fit.

Conversion factor

1 mU/L = 6 pmol/L = 0,035 µg/L = 35 pg/mL

Molecular weight of insulin lispro is: 5808 g/mol

Lispro NL-ELISA is calibrated against a highly purified, fully validated, commercial European Pharmacopoeia Reference Standards: Insulin Lispro CRS4.

Typical Standard Curve

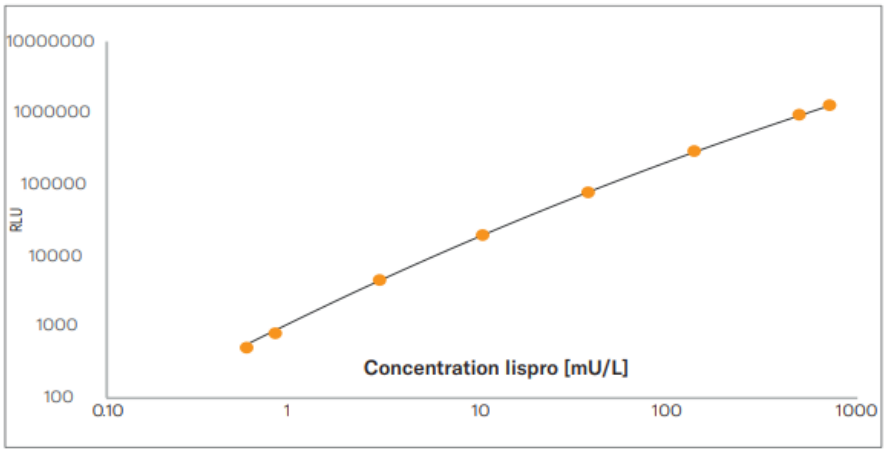
These values were obtained using BMG Labtech Clariostar with 1 s integration time, 2500 gain and 11.5 mm focal height.

Wells	Identity	Mean RLU**	Mean conc. mU/L
1 A-D	Calibrator 0	66	<Anchor Point Low
	Anchor Point Low*	649	0.71
1 E-H	Calibrator 1*	1109	1.06
2 A-D	Calibrator 2*	4887	3.38
2 E-H	Calibrator 3*	21279	11.6
3 A-D	Calibrator 4*	81047	38.8
3 E-H	Calibrator 5*	306242	143
4 A-D	Calibrator 6*	973581	495
4 E-H	Anchor Point High*	1327796	704
8 A-D	Control Low*	2524	2.0
8 E-H	Control Medium*	46396	23.2
9 A-D	Control High*	806775	401

*Concentration stated on vial label.

**The RLU is affected by reader model and gain settings. Different readers can give different RLU values. Absolute RLU values do not affect calculated results.

A typical calibrator curve is shown here. Do not use this curve to determine actual assay results.



Reference Values

Good practice dictates that each laboratory establishes its own expected range of values.

Performance Characteristics

High dose hook effect

Samples with a concentration up to 10 000 mU/L (20X Cal 6) can be measured without giving falsely low results.

Precision

1. Human serum

Human serum controls spiked with lispro were analyzed in 4 replicates on at least 12 different occasions by 2 different technicians on 4 days.

Sample	Mean value mU/L	Between run-Accuracy %	Coefficient of variation	
			Repeatability %*	Within laboratory %**
QC_{LLOQ}	0.94	94	6.7	7.7
QC_{Low}	1.89	95	3.5	5.8
QC_{Medium}	21.4	86	2.2	3.7
QC_{High}	364	91	1.9	3.0
QC_{ULOQ}	459	92	2.5	3.4

*Within assay variation

**Total assay variation

2. Human EDTA Plasma

Human EDTA plasma, human P-800 plasma and porcine EDTA plasma were spiked with EDQM Lispro and analyzed in 4 replicates on 6 occasions by 2 different technicians on 2 days.

Sample	Mean value mU/L	Between run-Accuracy %	Coefficient of variation	
			Repeatability %*	Within laboratory %**
QC_{LLOQ}	0.98	98	2.4	3.6
QC_{Low}	1.95	98	3.7	5.2
QC_{Medium}	22.5	90	2.1	4.0
QC_{High}	333	83	2.1	3.2
QC_{ULOQ}	415	83	3.5	4.7

*Within assay variation

**Total assay variation

3. Human P-800 Plasma

Sample	Mean value mU/L	Between run-Accuracy %	Coefficient of variation	
			Repeatability %*	Within laboratory %**
QC_{LLOQ}	1.07	107	3.3	3.4
QC_{Low}	2.08	104	1.3	4.1
QC_{Medium}	22.2	89	1.4	2.7
QC_{High}	336	84	1.1	1.7
QC_{ULOQ}	417	83	1.4	3.5

*Within assay variation

**Total assay variation

4. Porcine EDTA Plasma

Sample	Mean value mU/L	Between run-Accuracy %	Coefficient of variation	
			Repeatability %*	Within laboratory %**
QC_{LLOQ}	1.09	109	2.1	4.3
QC_{Low}	2.00	100	1.8	3.85
QC_{Medium}	22.3	89	2.1	2.96
QC_{High}	332	83	0.8	1.9
QC_{ULOQ}	424	85	1.7	3.41

*Within assay variation

**Total assay variation

Detection Limit

Capability of Detection is 0.36 mU/L as determined by the methodology described.

Lower Limit of Quantification, LLOQ, is 1.0 mU/L as determined according to EMA/FDA guidelines.

The Upper Limit of Quantification, ULOQ, is 500 mU/L as determined according to EMA/FDA guidelines.

Sensitivity

Selectivity has been validated according to FDA/EMA guidelines. At least 10 blank samples from different individuals of each tested matrix (human serum, human EDTA plasma, human P-800 plasma and porcine

EDTA-plasma) showed no positive signal in the assay in the absence of lispro. Auto-insulin antibodies, HAMA, rheumatoid factor (RF), grossly lipemic, icteric or hemolyzed samples do not interfere in the assay.

Specificity

The following cross-reactions and interferences were studied:

Substance	Concentrations tested	Cross-reaction	Interference (Acceptence criteria 100 ± 25 %) LLOQ (Recovery %)	ULOQ (Recovery %)
Native Human Insulin	50-400 mU/L	N.D.	105-112	97-114
Native Human Proinsulin	50-300 pmol/L	N.D.	104-113	103-110
Glargine	50-600 mU/L	N.D.	96-117	89-107
Glargine M1	50-600 mU/L	N.D.	98-105	100-107
Glargine M2	50-600 mU/L	N.D.	104-123	109-115
Degludec	50-600 mU/L	N.D.	101-113	103-109
Detemir	50-600 mU/L	N.D.	107-116	108-110
Insulin NPH	50-600 mU/L	N.D.	106-116	102-108
Aspart	50-600 mU/L	N.D.	95-111	101-106
Glulisine	50-600 mU/L	N.D.	101-112	99-103
Native Porcine Insulin	50-600 mU/L	N.D.	-	-
Native Porcine Proinsulin	50-300 pmol/L	N.D.	-	-

N.D. = Not Detected

Limitations

The assay is specific for insulin lispro with no cross-reactivity to any of the tested insulin analogs, insulin autoantibodies, native insulin and native proinsulin as shown in performance characteristics.