Erenna®

Insulin Mouse Immunoassay Kit

384-Well Plate Assay

Catalog #03-0006-03

Immunoassay kit for the quantitative determination of **Mouse Insulin** in heparin plasma

FOR RESEARCH USE ONLY

NOT INTENDED FOR DIAGNOSTIC OR THERAPEUTIC PROCEDURES

Manufactured & Distributed by:

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INTRODUCTION

The Erenna® Insulin Mouse Immunoassay Kit uses a quantitative fluorescent sandwich immunoassay technique to measure insulin in mouse heparin plasma samples. A capture antibody specific for mouse insulin has been pre-coated onto a 384-well microplate. The user pipettes standards and samples into microplate wells along with a fluorescent-labeled dye detection antibody. During incubation, the insulin present in the sample binds to the solid phase capture antibody. Unbound insulin molecules are washed away during the subsequent wash step. This detection antibody will recognize and bind to insulin that has been captured onto the solid phase. After washing, elution buffer is added and incubated to dissociate the bound protein sandwiches from the plate surface. The fluorescent antibodies are now free-floating in the wells. The plate is loaded into the Erenna System and the fluorescent molecules are counted. The number of fluorescently labeled detection antibodies counted is directly proportional to the amount of insulin that was present in the sample when captured. The amount of insulin in unknown samples is interpolated off of the standard curve.

MATERIALS

The Erenna® Total GLP-1 Immunoassay kit includes all reagents listed in Table 1: Reagent Data. Additional reagents and supplies may be required to run this immunoassay, as listed in APPENDIX B: Additional Supplies Required.

Reagents Provided

Item #	Description	Shipping Conditions	Storage Conditions	Component Part No.
1	Mouse Insulin Assay Plate	With cold pack	2–8°C	02-0123-00
2	Mouse Insulin Detection Reagent	With cold pack	2–8°C	02-0124-01
3	Mouse Insulin Standard (frozen, shipped in separate box)	On dry ice	≤ -70°C	02-0121-01
4	Mouse Insulin Standard Diluent	With cold pack	2–8°C	02-0122-01
5	10X Wash Buffer	With cold pack	Ambient	02-0001-00
6	Elution Buffer (insulin free)	With cold pack	2-8°C	02-0126-01
7	Erenna® Insulin Mouse Immunoassay Kit Instructions	N/A	Ambient	05-0122-05

Table 1: Reagent Data

Storage Instructions

The **Erenna® Mouse Insulin** Immunoassay Reagent Kit should be stored at 2–8°C. The Standard analyte should be stored at ≤ -70°C. Proper kit performance can only be guaranteed if the materials are stored properly.

General Supplies Required (manufacturer not specified)

- De-ionized or distilled water
- 96-well polypropylene micro-plate
- Multi-channel pipette capable of transferring 20 µL, 30 µL and 90 µL
- Micro-centrifuge tube
- Mini-centrifuge
- Microplate centrifuge
- 1L container and 1L graduated cylinder
- 384-well microplate washer

ASSAY PREPARATION

Reagent Preparation

1. Warm the following reagents to room temperature prior to use: **Standard Diluent, 10x Wash Buffer**, and **Elution Buffer (insulin free)**.
2. Place the following reagents at 4°C until ready to use: **Mouse Insulin Detection Reagent**.
3. Prepare 1X Wash Buffer (from 10X Wash Buffer) as follows:
    a. Pour the contents of the 100 mL bottle of 10X Wash Buffer into a container capable of holding at least 1L
    b. Add 900 mL of deionized water
    c. Mix thoroughly by gentle inversion or with a clean, sterile stir bar

Sample Preparation

1. Prepare samples by one of the following methods:
    a. If using a filter plate with prefilter (Pall PN: 5041): Stack the Pall 5041 filter plate on top of a 96-well receptacle plate. Place 250 µL of sample into a filter plate well and spin for ≥ 10 minutes at 1,100 x *g*.
    b. If using a microcentrifuge: Centrifuge heparin plasma samples at >13,000 x *g* for 10 minutes immediately prior to use. Carefully pipette the supernatant into a clean microcentrifuge tube, avoiding particulates and slowly aspirating below the lipid layer. Avoid repeated freeze–thaw cycles. Add samples to the 96-well plate for ease in transferring.

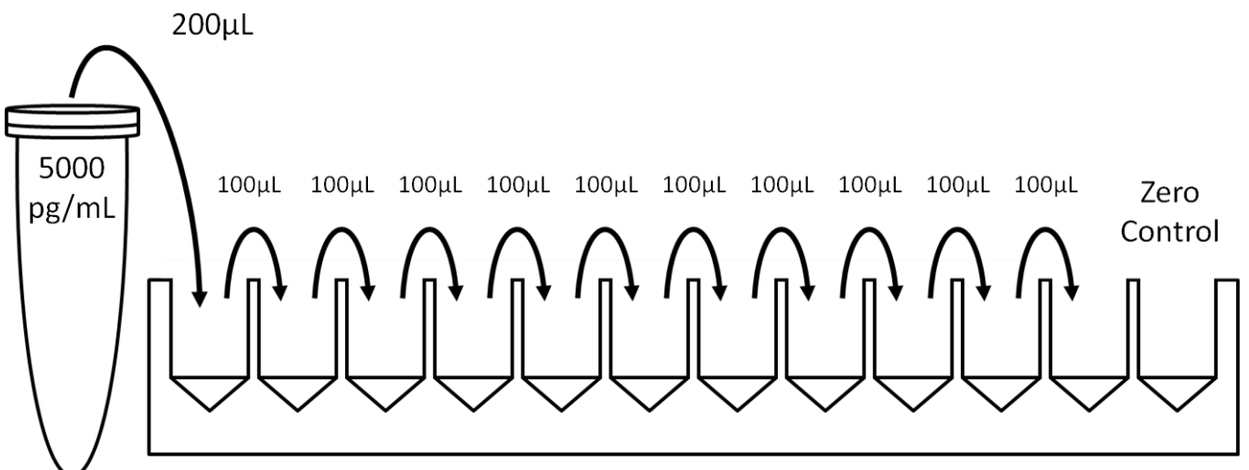
Initial Standard Stock Preparation

1. Vortex, quick spin and pipette mix the **Mouse Insulin Standard Analyte** vial in a mini-centrifuge prior to opening. Use care when opening this concentrated standard vial to prevent loss of materials and contamination of specimens or plates with aerosols.
2. Refer to the standard value assignment for the starting concentration of the **Mouse Insulin Standard Analyte** in the vial.To make your **Analyte Working Stock**, perform the necessary dilutions to achieve the final working concentration of 5 ng/mL (or 5000 pg/mL) of at least a 200 µL final volume.

MOUSE INSULIN ASSAY PROCEDURE

Standard Curve

Prepare the standard curve in a 12-channel reservoir dilution plate. Perform 1:2 serial dilutions of the **Analyte Working Stock** to achieve a curve from 5000 pg/mL to 4.9 pg/mL.



1	2	3	4	5	6	7	8	9	10	11	12
5,000	2,500	1,250	625	313	156	78	39	19.5	9.8	4.9	0

1. Add 100 µL **Standard Diluent** to wells 2 through 12 of a 12-channel reservoir dilution plate.
2. Add 200 µL of the 5000 pg/mL **Analyte Working Stock** from standard preparation into well 1.
3. Transfer 100 µL from well 1 into well 2, mixing thoroughly. Continue serial dilutions from well 2 stopping at well 11. **Use a fresh tip with each transfer**.

Assay Set-Up

1. Prepare reagents according to Reagent Preparation section above.
2. Prepare the standard and the standard curve according to the Assay Preparation section.
3. Prepare the samples according to Sample Preparation section.
4. Add Detection Reagent to 96-well polypropylene plate (90 µL per well).
5. Add Standard curve and samples (30 µL per well) to 96-well polypropylene plate already containing Detection Reagent. Mix on shaker for 5 minutes.
6. Transfer sample mix from 96-well plate to 384-well assay plate (20 µL per well). It is recommended to run standards and samples in quadruplicate.

Target Capture

1. Cover plate with an Axyseal plate cover. Carefully avoid splashing.
2. Centrifuge plate to ensure that all liquid is at the bottom of well (1 minute at 3,000 RPM).
3. Incubate for 2 hours at 25°C on Boekel Scientific, The Jitterbug™ setting 5.
4. When incubation is complete, carefully remove temporary plate cover to avoid splashing.
5. Wash plate 6 times with1X wash buffer using 100 µL per well.
6. Centrifuge plate inverted onto a paper towel to remove excess liquid (1 minute at 3,000 RPM).

Elution

1. Add Elution Buffer (insulin free) to 384-well assay plate (20 µL per well).
2. Cover plate with Heat Sealing Foil, according to manufacturer instructions for the heat sealer.
3. Centrifuge plate to ensure all liquid is at the bottom of the well (1 minute at 3,000 RPM).
4. Incubate while shaking (1.5 hours at 25°C).

Run on Erenna Immunoassay System

1. Load completed assay **Plate** onto the Erenna Immunoassay System.

TECHNICAL HINTS DUE TO HIGH SENSITIVITY

- Wipe down bench and pipettes with 70% isopropanol before use.
- Quickly spin concentrated standard before opening vials.
- Use sterile filter pipette tips and reagent trays to avoid contamination.
- Use filter tips while transferring concentrated standard.
- Use a 12-channel reservoir for preparing standards.
- Pre-wet tips (aspirate and dispense within well) twice before each transfer. It is recommended to use a 96-well dilution (polypropylene) plate for preparing standards and samples.
- It is recommended to transfer four replicates of each standard point from the dilution plate into the 384-well Insulin Assay Plate.

ADDITIONAL SAMPLE INFORMATION

- The Erenna® Mouse Insulin Immunoassay has been validated using heparin plasma.
- Ensure sample is clear of precipitants and other visible particulate matter before testing with the Erenna® Mouse Insulin Immunoassay.

PRECAUTIONS

- Use caution when handling biological samples; wear protective clothing and gloves.
- Components of this reagent kit contain approximately 0.1% sodium azide as a preservative. Sodium azide is a toxic and dangerous compound when combined with acids or metals. Solutions containing sodium azide should be disposed of properly.

APPENDIX A: Erenna Plate Immunoassay Quick Guide

Insulin Quick Assay Guide

1. Prepare all reagents, standard curve, and samples as instructed.
2. Add 90 µL Detection Reagent to each well in dilution plate.
3. Add 30 µL Standard/Samples to dilution plate. Mix 5 minutes.
4. Transfer 20 µL of mixture to 384-well assay plate.
5. Cover and centrifuge assay plate. Incubate while shaking for 2 hours at RT.
6. Wash plate 6X; invert and centrifuge.
7. Add 20 µL Elution Buffer to each well.
8. Seal plate, centrifuge and incubate while shaking for 1.5 hours at RT.
9. Load plate onto the Erenna.

APPENDIX B: Additional Supplies Required (not provided)

Description	Mfr Supplier	Component Part Numbers	Product Uses	Packaging Detail
Erenna® 10X Systems Buffer	Singulex	02-0111-00, 02-0111-01	Systems (Analysis) Buffer, fluid used to run Erenna System	1L (10L mixed) 2L (20L mixed)
Reservoirs for 12-Channel Pipetters	VWR	80092-466	Standard Curve	10/pkg
96-Well Deep Well PP Plate (2.2 mL, 1.64 mL or 1.09 mL)	Axygen	P-2ML-SQ-C, P-DW-20-C or P-DW-11-C	Prepare standard curves (choose size)	Variable
8-Well Low Profile Reservoir	VWR	12000-732	Transfer of Reagents	Variable
384-Well Round Bottom PolyPropylene Plate, 120 µL	Nunc	264573	Receiver/analysis plate	20/pk or 120/cs
Syringe (5 ml)	VWR	66064-772 (or equivalent)	To filter diluted detection antibody	100 units/pk
0.2 µm Syringe Filter	Pall	4187	To filter diluted detection antibody	50/pk
AcroPrep™ 96-well Filter Plate (Supor Membrane)	Pall	5041	Alternate sample preparation	10/pkg
Universal Plate Cover	Nunc	253623	Cover the plate	25 units/pk
AxySeal—PCRSP Plate sealing film series	Axygen	PCR-SP	Sealing plates during incubation/ mix/store	100 films/ case
Microplate Wash Station	- - -	- - -	Washing	- - -
Centrifuge w/ Plate Rotor	- - -	- - -	Remove MP via filter plate ~1,100 xg	1
Microplate Incubator / Shaker	Boekel Scientific	130000 The Jitterbug™	Incubating plate	1
Heat Sealing Plate Foil	Singulex	01-0216-00 or equivalent	Sealing plate for analysis on Erenna	- - -
Heat Sealer with adjustable temperature and time	FluidX	XTS-384 or equivalent	Sealing plate for analysis on Erenna	1
Universal Adapter for SBS format plates	FluidX	42-1001 or equivalent	Required for proper sealing on XTS-384	1

CONTACT INFORMATION

To reach Singulex, Inc. reagent technical support, call **(510) 995-9000**, or in the U.S. you may call us toll-free at (888) 603-3033.

You can also send us an e-mail at **techsupport@singulex.com**

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