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Product Information

Mouse IGF-I ELISA Kit
for serum, plasma, and cell culture supernatant

Catalog Number **RAB0229**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

The Mouse IGF-I ELISA (Enzyme-Linked Immunosorbent Assay) kit is an *in vitro* enzyme-linked immunosorbent assay for the quantitative measurement of mouse IGF-I in serum, plasma, and cell culture supernatants. This assay employs an antibody specific for mouse IGF-I coated on a 96 well plate. Standards and samples are pipetted into the wells and IGF-I present in a sample is bound to the wells by the immobilized antibody. The wells are washed and biotinylated anti-mouse IGF-I antibody is added. After washing away unbound biotinylated antibody, HRP-conjugated streptavidin is pipetted to the wells. The wells are again washed, a TMB substrate solution is added to the wells and color develops in proportion to the amount of IGF-I bound. The Stop Solution changes the color from blue to yellow, and the intensity of the color is measured at 450 nm.

Components

1. Mouse IGF-I Antibody-coated ELISA Plate (Item A) - RAB0229A: 96 wells (12 strips × 8 wells) coated with anti-mouse IGF-I.
2. 20x Wash Buffer (Item B) - RABWASH4: 25 mL of 20x concentrated solution
3. Lyophilized Mouse IGF-I Protein Standard (Item C) - RAB0229C: 2 vials of recombinant mouse IGF-I.
4. ELISA 1x Assay/Sample Diluent Buffer A (Item D1) - RABELADA: 30 mL diluent buffer, 0.09% sodium azide as preservative. For Standard/Sample (serum/plasma) diluent.
5. ELISA 5x Assay/Sample Diluent Buffer B (Item E1) - RABELADB: 15 mL of 5x concentrated buffer. For Standard/Sample (cell culture medium) diluent.
6. Biotinylated Mouse IGF-I Detection Antibody (Item F) - RAB0229D: 2 vials of biotinylated anti-mouse IGF-I (each vial is enough to assay half microplate).
7. HRP-Streptavidin (Item G) - RABHRP5: 200 μL of 200x concentrated HRP-conjugated streptavidin.
8. ELISA Colorimetric TMB Reagent (HRP Substrate, Item H) - RABTMB3: 12 mL of 3,3′,5,5′-tetra-methylbenzidine (TMB) in buffer solution.
9. ELISA Stop Solution (Item I) - RABSTOP3: 8 mL of 0.2 M sulfuric acid.

Reagents and Equipment Required but Not Provided.

1. Microplate reader capable of measuring absorbance at 450 nm
2. Precision pipettes to deliver 2 μL to 1 mL volumes
3. Adjustable 1-25 mL pipettes for reagent preparation
4. 100 mL and 1 liter graduated cylinders
5. Absorbent paper
6. Distilled or deionized water
7. Log-log graph paper or computer and software for ELISA data analysis
8. Tubes to prepare standard or sample dilutions

Precautions and Disclaimer

This product is for Research Use Only. Not for Use in Diagnostic Procedures. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

1. Bring all reagents and samples to room temperature (18–25 °C) before use.

2. Sample dilution: Assay Diluent A (Item D) should be used for dilution of serum/plasma samples. 1x Assay Diluent B (Item E) should be used for dilution of cell culture supernatants. The suggested dilution for normal serum/plasma is 100 to 5,000-fold.

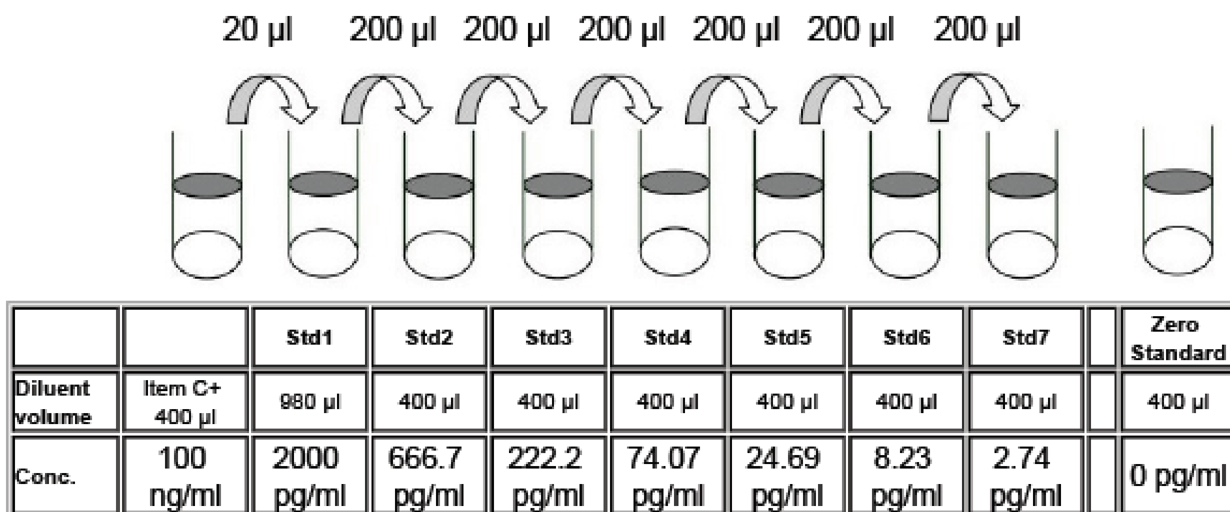
   Note: Levels of IGF-I may vary between different samples. Optimal dilution factors for each sample must be determined by the investigator.

3. Assay Diluent B should be diluted 5-fold with deionized or distilled water before use.

4. Preparation of standard: **Briefly spin the vial of Item C** and then add 400 μL of Assay Diluent A (for serum/plasma samples) or 1x Assay Diluent B (for cell culture supernatants) into Item C vial to prepare a 100 ng/mL standard. **Dissolve the powder thoroughly by a gentle mix.**

   Add 20 μL of IGF-I standard (100 ng/mL) from the vial of Item C into a tube with 980 μL of Assay Diluent A or 1x Assay Diluent B to prepare a 2,000 pg/mL standard solution. Pipette 400 μL of Assay Diluent A or 1x Assay Diluent B into each tube. Use the 2,000 pg/mL standard solution to produce a dilution series (Figure 1). Mix each tube thoroughly before the next transfer. Assay Diluent A or 1x Assay Diluent B serves as the zero standard (0 ng/mL).

Figure 1.
Dilution Series for Standards



		Std1	Std2	Std3	Std4	Std5	Std6	Std7		Zero Standard
Diluent volume	Item C+ 400 µl	980 µl	400 µl	400 µl	400 µl	400 µl	400 µl	400 µl		400 µl
Conc.	100 ng/ml	2000 pg/ml	666.7 pg/ml	222.2 pg/ml	74.07 pg/ml	24.69 pg/ml	8.23 pg/ml	2.74 pg/ml		0 pg/ml

5. If the Wash Concentrate (20x) (Item B) contains visible crystals, warm to room temperature and mix gently until dissolved. Dilute 20 mL of Wash Buffer Concentrate into deionized or distilled water to yield 400 mL of 1x Wash Buffer.

6. Briefly spin the Detection Antibody vial (Item F) before use. Add 100 μL of 1x Assay Diluent B into the vial to prepare a detection antibody concentrate. Pipette up and down to mix gently (the concentrate can be stored at 4 °C for 5 days). The detection antibody concentrate should be diluted 80-fold with 1x Assay Diluent B and used in Procedure, step 5.

7. Briefly spin the HRP-Streptavidin concentrate vial (Item G) and pipette up and down to mix gently before use. HRP-Streptavidin concentrate should be diluted 200-fold with 1x Assay Diluent B.

   For example: Briefly spin the vial (Item G) and pipette up and down to mix gently. Add 50 μL of HRP-Streptavidin concentrate into a tube with 10 mL of 1x Assay Diluent B to prepare a 200-fold diluted HRP-Streptavidin solution (don't store the diluted solution for next day use). Mix well.

Storage/Stability

Store the kit at –20 °C. It remains active for up to 1 year. Avoid repeated freeze-thaw cycles.

The reconstituted standard should be stored at –20 °C or –70 °C (–70 °C is recommended). Opened microplate strips or reagents may be store for up to 1 month at 2–8 °C. Return unused wells to the pouch containing desiccant pack and reseal along entire edge.

Procedure

1. Bring all reagents and samples to room temperature (18–25 °C) before use. It is recommended that all standards and samples be run at least in duplicate.

2. Label removable 8 well strips as appropriate for the experiment.

3. Add 100 μL of each standard (see Preparation, step 4) and sample into appropriate wells. Cover well and incubate for 2.5 hours at room temperature or over night at 4 °C with gentle shaking.

4. Discard the solution and wash 4 times with 1x Wash Solution. Wash by filling each well with Wash Buffer (300 μL) using a multichannel pipette or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.

5. Add 100 μL of 1x prepared biotinylated antibody (see Preparation, step 6) to each well. Incubate for 1 hour at room temperature with gentle shaking.

6. Discard the solution. Repeat the wash as in step 4.

7. Add 100 μL of prepared Streptavidin solution (see Preparation, step 7) to each well. Incubate for 45 minutes at room temperature with gentle shaking.

8. Discard the solution. Repeat the wash as in step 4.

9. Add 100 μL of TMB One-Step Substrate Reagent (Item H) to each well. Incubate for 30 minutes at room temperature in the dark with gentle shaking.

10. Add 50 μL of Stop Solution (Item I) to each well. Read at 450 nm immediately.

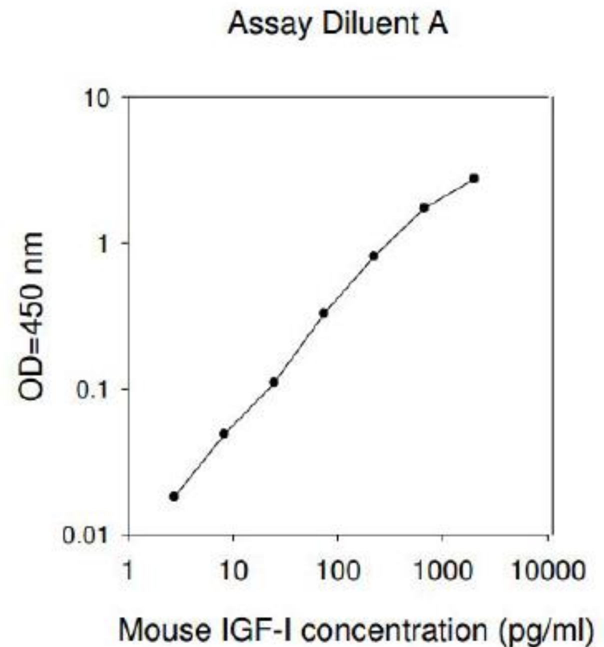
Results

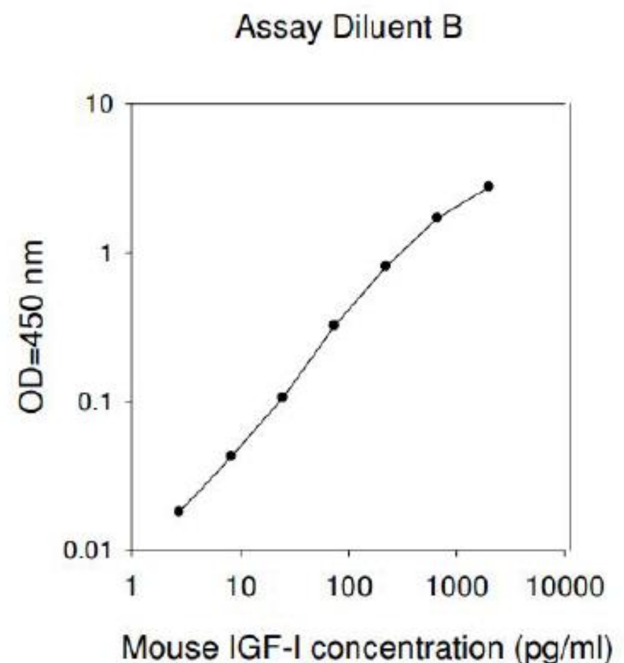
Calculations

Calculate the mean absorbance for each set of duplicate standards, controls and samples, and subtract the average zero standard optical density. Plot the standard curve on log-log graph paper or using Sigma plot software, with standard concentration on the x-axis and absorbance on the y-axis. Draw the best-fit straight line through the standard points.

Typical Data

These standard curves are for demonstration only. A standard curve must be run with each assay.





Product Profile

Sensitivity: The minimum detectable dose of Mouse IGF-I was determined to be 4 pg/ml.

Minimum detectable dose is defined as the analyte concentration resulting in an absorbance that is 2 standard deviations higher than that of the blank (diluent buffer).

Reproducibility:

Intra-Assay: CV <10%
Inter-Assay: CV <12%

Spiking & Recovery: Recovery was determined by spiking various levels mouse IGF-I into mouse serum, plasma, and cell culture media. Mean recoveries are as follows:

Sample Type	Average % Recovery	Range (%)
Serum	101.2	85-117
Plasma	106.6	85-114
Cell culture media	86.40	72-99

Linearity:

Sample Type		Serum	Plasma	Cell Culture Media
1:2	Average % of Expected	127.3	124.4	127.0
	Range (%)	116-134	116-130	120-132
1:4	Average % of Expected	119.1	113.5	132.7
	Range (%)	110-28	105-121	127-142

Specificity

Cross Reactivity: This ELISA kit shows no cross-reactivity with the following cytokines tested: Mouse CD30, LCD30, T CD40, CRG-2, CTACK, CXCL16, Eotaxin , Eotaxin-2, Fas Ligand, Fractalkine, GCSF, GM-CFS, IFN-gamma, IGFBP-3, IGFBP-5, IGFBP-6, IL-1alpha, IL-1beta, IL-2, IL-3, IL-3 Rb, IL-4, IL-5, IL-9, IL-10, IL-12 p40/p70, IL-12 p70, IL-13, IL-17, KC, Leptin R, LEPTIN(OB), LIX, L-Selectin, Lymphotactin, MCP-1, MCP- 5, M-CSF, MIG, MIP-1alpha, MIP-1gamma, MIP-2, MIP-3beta, MIP-3alpha, PF-4, P-Selectin, RANTES, SCF, SDF-1alpha,TARC, TCA-3, TECK, TIMP-1, TNF-alpha, TNF RI, TNF RII, TPO, VCAM-1, VEGF.

Appendix

Troubleshooting Guide

Problem	Cause	Solution
Poor standard curve	Inaccurate pipetting	Check pipettes
	Improper standard dilution	Ensure a brief spin of Item C and dissolve the powder thoroughly with gentle mixing.
	Improper preparation of standard and/or biotinylated antibody	Briefly spin down vials before opening. Dissolve the powder thoroughly.
Low signal	Too brief incubation times	Ensure sufficient incubation time; Procedure, step 3 may be done over night at 4 °C with gentle shaking. Note: May increase overall signals including background.
	Inadequate reagent volumes or improper dilution	Check pipettes and ensure correct preparation
Large CV	Inaccurate pipetting	Check pipettes
	Air bubbles in wells	Remove bubbles in wells
High background	Plate is insufficiently washed	Review the manual for proper wash. If using a plate washer, ensure that all ports are unobstructed.
	Contaminated wash buffer	Make fresh wash buffer
Low sensitivity	Improper storage of the ELISA kit	Store the standard at <–20 °C after reconstitution, others at 4 °C. Keep substrate solution protected from light
	Stop solution	Add stop solution to each well before reading plate.

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