Igf1 (Mouse) ELISA Kit

Catalog Number KA0493

96 assays

Version: 14

Intended for research use only

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Introduction

Background

Insulin-like growth factor 1 (IGF-1, somatomedin) is a 70 amino acid polypeptide protein hormone with molecular mass of 7.65 kDa (1). IGF-1 is produced primarily by the liver in response to the stimulation of growth hormone. It is transported in plasma bound to different forms of IGF-1 binding proteins (2). It also binds to specific IGF-1 tyrosine kinase receptor and the insulin receptor. Inhibition IGF-1 receptor reduces pancreatic cancer growth and angiogenesis (3). IGF-I regulates cellular proliferation, differentiation, apoptosis, and amyloid precursor protein family (4, 5). It may be important in the pathophysiological processes underlying chronic disease, including type 2 diabetes mellitus, coronary heart disease, cancer, and Alzheimer's disease (6-8). Increased levels of IGF lead to an increase risk of cancer (9). IGF-I stimulates osteoblast proliferation, bone formation, and increases bone volume (9). IGF-1 stimulates osteoblast proliferation, bone formation, and increases bone volume (10). It is a potent neurotrophic as well as a neuroprotective factor found in the central and the peripheral nervous systems of the brain (11).

Principle of the Assay

The Igf1 (Mouse) ELISA Kit is designed for detection of IGF-1 in mouse plasma, serum and cell culture samples. This assay employs a quantitative sandwich enzyme immunoassay technique that measures mouse IGF-1 in approximately 5 hours. A polyclonal antibody specific for mouse IGF-1 has been pre-coated onto a 96-well microplate with removable strips. IGF-1 in standards and samples is sandwiched by the immobilized antibody and a biotinylated polyclonal antibody specific for mouse IGF-1, which is recognized by a streptavidin-peroxidase (SP) conjugate. All unbound material is washed away and a peroxidase enzyme substrate is added. The color development is stopped and the intensity of the color is measured

General Information

Materials Supplied

List of component

Component	Amount
Mouse IGF-1 Microplate: A 96 well polystyrene microplate coated with a polyclonal antibody against mouse IGF-1.	96 (8x12) well
Sealing Tapes: Pressure sensitive sealing tapes that can be cut to fit the format of the individual assay.	3 slices
Mouse IGF-1 Standard: Mouse IGF-1 in a buffered protein base (lyophilized).	40 ng
Biotinylated Mouse IGF-1 Antibody (40x): A 40-fold concentrated biotinylated polyclonal antibody against mouse IGF-1.	150 µL
MIX Diluent Concentrate (10x): A 10-fold concentrated buffered protein base.	30 mL
Wash Buffer Concentrate (20x): A 20-fold concentrated buffered surfactant.	30 mL x 2
SP Conjugate (100x): A 100-fold concentrate.	80 µL
Chromogen Substrate (1x): A stabilized peroxidase chromogen substrate tetramethylbenzidine.	8 mL
Stop Solution (1x): A 0.5 N hydrochloric acid solution to stop the chromogen substrate reaction.	12 mL

Storage Instruction

- ✓ Upon arrival, immediately store components of the kit at recommended temperatures up to the expiration date.
- ✓ Store SP Conjugate and Biotinylated Antibody at -20°C.
- ✓ Store Microplate, Diluent Concentrate (10x), Wash Buffer, Stop Solution, and Chromogen Substrate at 2-8°C.
- ✓ Unused microplate wells may be returned to the foil pouch with the desiccant packs and resealed. May be stored for up to 30 days in a vacuum desiccator.
- ✓ Store standard at 2-8°C before reconstituting with Diluent and at -20°C after reconstituting with Diluent.

Materials Required but Not Supplied

- ✓ Microplate reader capable of measuring absorbance at 450 nm.
- ✓ Pipettes (1-20 µL, 20-200 µL, 200-1000 µL and multiple channels).
- ✓ Deionized or distilled reagent grade water

Precautions for Use

- ✓ This product is for Research Use Only and is not intended for use in diagnostic procedures.
- ✓ Prepare all reagents (diluent buffer, wash buffer, standard, biotinylated antibody, and SP conjugate) as instructed, prior to running the assay.
- ✓ Prepare all samples prior to running the assay. The dilution factors for the samples are suggested in this insert. However, the user should determine the optimal dilution factor.
- ✓ Spin down the SP conjugate vial and the biotinylated antibody vial before opening and using contents.
- ✓ The Stop Solution is an acidic solution.
- ✓ The kit should not be used beyond the expiration date.

Assay Protocol

Reagent Preparation

Freshly dilute all reagents and bring all reagents to room temperature before use.

- ✓ MIX Diluent Concentrate (10x): If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved. Dilute the MIX Diluent Concentrate 10-fold with reagent grade water to produce a 1x solution. Store for up to 30 days at 2 - 8°C.
- ✓ Mouse IGF-1 Standard: Reconstitute the Mouse IGF-1 Standard (40 ng) with 4 mL of MIX Diluent to generate a 10 ng/mL standard stock solution. Allow the vial to sit for 10 minutes with gentle agitation prior to making dilutions. Prepare duplicate or triplicate standard points by serially diluting from the standard stock solution (10 ng/mL) 2-fold with equal volume of MIX Diluent to produce 5, 2.5, 1.25, 0.625, 0.313 and 0.156 ng/mL solutions. MIX Diluent serves as the zero standard (0 ng/mL). Any remaining stock solution should be stored at -20°C and used within 30 days. Avoid repeated freeze-thaw cycles.

Standard Point	Dilution	[IGF-1] (ng/mL)
P1	1 part Standard (10 ng/mL)	10.00
P2	1 part P1 + 1 part MIX Diluent	5.000
P3	1 part P2 + 1 part MIX Diluent	2.500
P4	1 part P3 + 1 part MIX Diluent	1.250
P5	1 part P4 + 1 part MIX Diluent	0.625
P6	1 part P5 + 1 part MIX Diluent	0.313
P7	1 part P6 + 1 part MIX Diluent	0.156
P8	MIX Diluent	0.000

- ✓ Biotinylated Mouse IGF-1 Antibody (40x): Spin down the antibody briefly and dilute the desired amount of the antibody 40-fold with MIX Diluent to produce a 1x solution. The undiluted antibody should be stored at -20°C.
- ✓ Wash Buffer Concentrate (20x): If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved. Dilute the Wash Buffer Concentrate 20-fold with reagent grade water to produce a 1x solution.
- ✓ SP Conjugate (100x): Spin down the SP Conjugate briefly and dilute the desired amount of the conjugate 100-fold with MIX Diluent to produce a 1x solution. The undiluted conjugate should be stored at -20°C.

Sample Preparation

- ✓ Plasma: Collect plasma using one-tenth volume of 0.1 M sodium citrate as an anticoagulant. Centrifuge samples at 3000 x g for 10 minutes and collect plasma. A 250-fold sample dilution is suggested into MIX Diluent; however, user should determine optimal dilution factor depending on application needs. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw

cycles.

- ✓ Serum: Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 3000 x g for 10 minutes and remove serum. A 250-fold sample dilution is suggested into MIX Diluent; however, user should determine optimal dilution factor depending on application needs. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.
- ✓ Cell Culture Supernatants: Centrifuge cell culture media at 1500 rpm for 10 minutes at 4°C to remove debris and collect supernatants. Samples can be stored at -80°C. Avoid repeated freeze-thaw cycles.

  Applicable samples may also include biofluids, cell culture, and tissue homogenates. If necessary, user should determine optimal dilution factor depending on application needs.
- ✓ Refer to Dilution Guidelines for further instruction.

Guidelines for Dilutions of 100-fold or Greater (for reference only; please follow the insert for specific dilution suggested)	
100x	10000x
A) 4 µL sample : 396 µL buffer (100x) = 100-fold dilution Assuming the needed volume is less than or equal to 400 µL.	A) 4 µL sample : 396 µL buffer (100x) B) 4 µL of A : 396 µL buffer (100x) = 10000-fold dilution Assuming the needed volume is less than or equal to 400 µL.
1000x	100000x
A) 4 µL sample : 396 µL buffer (100x) B) 24 µL of A : 216 µL buffer (10x) = 1000-fold dilution Assuming the needed volume is less than or equal to 240 µL.	A) 4 µL sample: 396 µL buffer (100x) B) 4 µL of A : 396 buffer (100x) C) 24 µL of B : 216 µL buffer (10x) = 100000-fold dilution Assuming the needed volume is less than or equal to 240 µL.

Assay Procedure

1. Prepare all reagents, standard solutions and samples as instructed. Bring all reagents to room temperature before use. The assay is performed at room temperature (20-25°C).
2. Remove excess microplate strips from the plate frame and return them immediately to the foil pouch with desiccant inside. Reseal the pouch securely to minimize exposure to water vapor and store in a vacuum desiccator.
3. Add 50 µL of Mouse IGF-1 Standard or sample to each well. Gently tap plate to thoroughly coat the wells. Break any bubbles that may have formed. Cover wells with a sealing tape and incubate for 2 hours. Start the timer after the last addition.
4. Wash the microplate manually or automatically using a microplate washer. Invert the plate and decant the contents; hit 4-5 times on absorbent material to completely remove the liquid. If washing manually, wash

five times with 200 µL of Wash Buffer per well. Invert the plate each time and decant the contents; hit 4-5 times on absorbent material to completely remove the liquid. If using a microplate washer, wash six times with 300 µL of Wash Buffer per well; invert the plate and hit 4-5 times on absorbent material to completely remove the liquid.

5. Add 50 µL of Biotinylated Mouse IGF-1 Antibody to each well. Gently tap plate to thoroughly coat the wells. Break any bubbles that may have formed. Cover wells with a sealing tape and incubate for 2 hours.
6. Wash a microplate as described above.
7. Add 50 µL of SP Conjugate to each well. Gently tap plate to thoroughly coat the wells. Break any bubbles that may have formed. Cover wells with a sealing tape and incubate for 30 minutes. Turn on the microplate reader and set up the program in advance.
8. Wash a microplate as described above.
9. Add 50 µL of Chromogen Substrate to each well. Gently tap plate to thoroughly coat the wells. Break any bubbles that may have formed. Incubate for 20 minutes or until the optimal blue color density develops.
10. Add 50 µL of Stop Solution to each well. The color will change from blue to yellow. Gently tap plate to ensure thoroughly mixing. Break any bubbles that may have formed.
11. Read the absorbance on a microplate reader at a wavelength of 450 nm immediately. If wavelength correction is available, subtract readings at 570 nm from those at 450 nm to correct optical imperfections. Otherwise, read the plate at 450 nm only. Please note that some unstable black particles may be generated at high concentration points after stopping the reaction for about 10 minutes, which will reduce the readings.

✓ Assay Summary

1. Add 50 µL of Standard or Samples per well. Incubate 2 hours.
2. Wash, then add 50 µL of Biotinylated Antibody per well. Incubate 2 hours.
3. Wash, then add 50 µL of SP Conjugate per well. Incubate 30 minutes.
4. Wash, then add 50 µL of Chromogen Substrate per well. Incubate 20 minutes.
5. Add 50 µL of Stop Solution per well. Read at 450 nm immediately.

Data Analysis

Calculation of Results

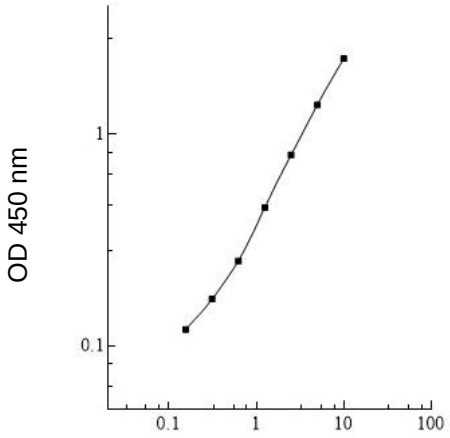
- ✓ Calculate the mean value of the duplicate or triplicate readings for each standard and sample.
- ✓ To generate a standard curve, plot the graph using the standard concentrations on the x-axis and the corresponding mean 450 nm absorbance (OD) on the y-axis. The best fit line can be determined by regression analysis using log-log or four-parameter logistic curve-fit.
- ✓ Determine the unknown sample concentration from the Standard Curve and multiply the value by the dilution factor.
- ✓ Typical Data

  The typical data is provided for reference only. Individual laboratory means may vary from the values listed. Variations between laboratories may be cause by technique differences.

Standard Point	ng/mL	OD	Average OD
P1	10.00	2.351 2.375	2.363
P2	5.000	1.427 1.417	1.422
P3	2.500	0.814 0.798	0.806
P4	1.250	0.464 0.456	0.406
P5	0.625	0.285 0.297	0.291
P6	0.313	0.208 0.216	0.212
P7	0.156	0.172 0.178	0.175
P8	0.000	0.132 0.132	0.132

✓ Typical Standard Curve for Igf1 (Mouse) ELISA Kit

The curve is provided for illustration only. A standard curve should be generated each time the assay is performed.



Performance Characteristics

✓ The minimum detectable dose of mouse IGF-1 as calculated by 2SD from the mean of a zero standard was established to be 0.12 ng/mL.

✓ Intra-assay precision was determined by testing three plasma samples twenty times in one assay.

✓ Inter-assay precision was determined by testing three plasma samples in twenty assays.

	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	20	20	20
CV (%)	3.6%	4.1%	3.9%	8.3%	8.5%	9.1%
Average CV (%)	3.9%			8.6%		

✓ Recovery

Standard Added Value	0.3 - 5.0 ng/mL
Recovery %	87 - 112%
Average Recovery %	96%

✓ Linearity

Plasma and serum samples were serially diluted to test for linearity

Average Percentage of Expected Value (%)		
Sample Dilution	Plasma	Serum
125x	94%	96%
250x	98%	101%
500x	105%	104%

✓ Cross-Reactivity

Species	Cross Reactivity (%)
Canine	None
Monkey	None
Rat	<70 %
Swine	<5%
Bovine	None
Rabbit	None
Human	<2 %

Resources

Troubleshooting

Issue	Causes	Course of Action
Low Precision	Use of Improper components	Check the expiration date listed before use. Do not interchange components from different lots.
	Improper wash step	Check that the correct wash buffer is being used. Check that all wells are empty after aspiration. Check that the microplate washer is dispensing properly. If washing by pipette, check for proper pipetting technique.
	Splashing of reagents while loading wells	Pipette properly in a controlled and careful manner.
	Inconsistent volumes loaded into wells	Pipette properly in a controlled and careful manner. Check pipette calibration. Check pipette for proper performance.
	Insufficient mixing of reagent dilutions	Thoroughly agitate the lyophilized components after reconstitution. Thoroughly mix dilutions.
	Improperly sealed microplate	Check the microplate pouch for proper sealing. Check that the microplate pouch has no punctures. Check that three desiccants are inside the microplate pouch prior to sealing.
Unexpectedly Low or High Signal Intensity	Microplate was left unattended between steps	Each step of the procedure should be performed uninterrupted.
	Omission of step	Consult the provided procedure for complete list of steps.
	Step performed in incorrect order	Consult the provided procedure for the correct order.
	Insufficient amount of reagents added to wells	Check pipette calibration. Check pipette for proper performance.
	Wash step was skipped	Consult the provided procedure for all wash steps.
	Improper wash buffer	Check that the correct wash buffer is being used.
	Improper reagent preparation	Consult reagent preparation section for the correct dilutions of all reagents.
	Insufficient or prolonged incubation periods	Consult the provided procedure for correct incubation time.

Deficient Standard Curve Fit	Non-optimal sample dilution	Sandwich ELISA: If samples generate OD values higher than the highest standard point (P1), dilute samples further and repeat the assay. Competitive ELISA: If samples generate OD values lower than the highest standard point (P1), dilute samples further and repeat the assay. User should determine the optimal dilution factor for samples.
	Contamination of reagents	A new tip must be used for each addition of different samples or reagents during the assay procedure.
	Contents of wells evaporate	Verify that the sealing film is firmly in place before placing the assay in the incubator or at room temperature.
	Improper pipetting	Pipette properly in a controlled and careful manner. Check pipette calibration. Check pipette for proper performance.
	Insufficient mixing of reagent dilutions	Thoroughly agitate the lyophilized components after reconstitution. Thoroughly mix dilutions.

References

1. Sandberg-Nordqvist AC et al. (1993) Cancer Res. 53: 2475-2478
2. Jones JI and Clemmons DR (1995) Endocr. Rev. 16: 3-34
3. Moser C. et al. (2008) Eur. J. Cancer 44: 1577-1586
4. Remacle-Bonnet MM et al. (2000) Cancer Res. 60: 2007-2017
5. Adlerz L et al. (2007) J. Biol. Chem. 282: 10203 -10209
6. Sandhu MS (2005) Endocr. Dev. 9:44-54
7. Sima AA and Li ZG (2006) Rev. Diabet. Stud. 3: 161-168
8. Butovsky O et al. (2006) Proc. Natl. Acad. Sci. U.S.A. 103: 11784-11789
9. Jo H et a. (2008) Mol Cell Biol. 28:4285-4299l
10. Spencer EM et al. (1991) Bone 12: 21-26
11. Gomez JM (2008) Endocr. Metab. Immune Disord Drug Targes 8: 143-151

Plate Layout

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