ab108874 – Insulin like Growth Factor 1 (IGF1) Mouse ELISA Kit

Instructions for Use

For the quantitative measurement of mouse Insulin like Growth Factor 1 (IGF1) in mouse plasma, serum and cell culture supernatant.

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

Version 5c Last Updated 8 February 2023

Table of Contents

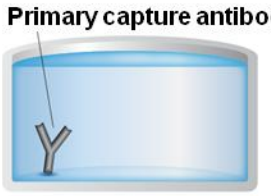
1. BACKGROUND

Abcam's Insulin like Growth Factor 1 (IGF1) Mouse *in vitro* ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed for the quantitative measurement of Insulin like Growth Factor 1 levels in plasma, serum and cell culture supernatant.

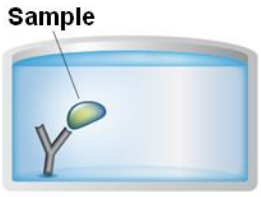
An IGF1 specific antibody has been precoated onto 96-well plates and blocked. Standards or test samples are added to the wells and subsequently an IGF1 specific biotinylated detection antibody is added and then followed by washing with wash buffer. Streptavidin-Peroxidase Conjugate is added and unbound conjugates are washed away with wash buffer. TMB is then used to visualize Streptavidin-Peroxidase enzymatic reaction. TMB is catalyzed by Streptavidin-Peroxidase to produce a blue color product that changes into yellow after adding acidic stop solution. The density of yellow coloration is directly proportional to the amount of IGF1 captured in plate.

Insulin-like growth factor 1 is a 70 amino acid polypeptide protein hormone with molecular mass of 7.65 kD. Insulin like Growth Factor 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 Insulin like Growth Factor 1 binding proteins. It also binds to specific Insulin like Growth Factor 1 tyrosine kinase receptor and the insulin receptor. Inhibition of Insulin like Growth Factor 1 receptor reduces pancreatic cancer growth and angiogenesis. Insulin like Growth Factor 1 regulates cellular proliferation, differentiation, apoptosis, and amyloid precursor protein family. It may be important in the pathophysiological processes underlying chronic disease, including type 2 diabetes mellitus, coronary heart disease, cancer, and Alzheimer's disease. Increased levels of IGF lead to an increased risk of cancer. Insulin like Growth Factor 1 stimulates osteoblast proliferation, bone formation, and increases bone volume. It is a potent neurotrophic as well as a neuroprotective factor found in the central and the peripheral nervous systems of the brain.

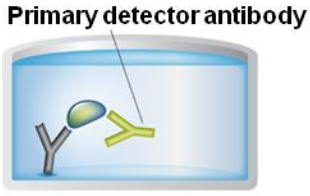
2. ASSAY SUMMARY



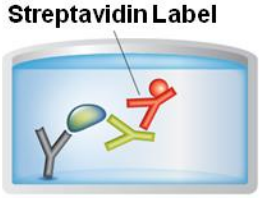
Prepare all reagents, samples and standards as instructed.



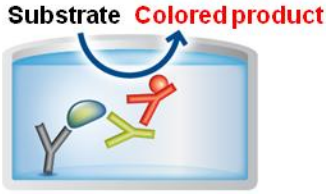
Add standard or sample to each well used. Incubate at room temperature.



Wash and add prepared biotin antibody to each well. Incubate at room temperature.



Wash and add prepared Streptavidin-Peroxidase Conjugate. Incubate at room temperature.



Add Chromogen Substrate to each well. Incubate at room temperature. Add Stop Solution to each well. Read immediately.

3. PRECAUTIONS

Please read these instructions carefully prior to beginning the assay.

Modifications to the kit components or procedures may result in loss of performance.

4. STORAGE AND STABILITY

Store kit at 4°C immediately upon receipt, apart from the Insulin like Growth Factor 1 Standard, SP Conjugate & Biotinylated Antibody, which should be stored at -20°C.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in sections 9 & 10.

5. MATERIALS SUPPLIED

Item	Amount	Storage Condition (Before Preparation)
Insulin like Growth Factor 1 Microplate (12 x 8 well strips)	96 wells	4°C
Insulin like Growth Factor 1 Standard	1 vial	-20°C
10X Diluent N Concentrate	30 mL	4°C
Biotinylated Mouse Insulin like Growth Factor 1 Antibody	1 vial	-20°C
100X Streptavidin-Peroxidase Conjugate (SP Conjugate)	80 µL	-20°C
1X Chromogen Substrate	7 mL	4°C
1X Stop Solution	11 mL	4°C
20X Wash Buffer Concentrate	2 x 30 mL	4°C
Sealing Tapes	3	N/A
Standard Diluent	2 mL	4°C

6. MATERIALS REQUIRED, NOT SUPPLIED

These materials are not included in the kit, but will be required to successfully utilize this assay:

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

7. LIMITATIONS

- Do not mix or substitute reagents or materials from other kit lots or vendors.

8. TECHNICAL HINTS

- Samples generating values higher than the highest standard should be further diluted in the appropriate sample dilution buffers.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Complete removal of all solutions and buffers during wash steps.
- **This kit is sold based on number of tests. A 'test' simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.**

9. REAGENT PREPARATION

Equilibrate all reagents to room temperature (18-25°C) prior to use. Prepare fresh reagents immediately prior to use. If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved.

9.1 **1X Diluent N**

Dilute the 10X Diluent N Concentrate 1:10 with reagent grade water to produce 1X Diluent N. Mix gently and thoroughly. *Store for up to 1 month at 4°C.*

9.2 **1X Wash Buffer**

If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved. Dilute the 20X Wash Buffer Concentrate 1:20 with reagent grade water to produce a 1X solution. Mix gently and thoroughly.

9.3 **1X Streptavidin-Peroxidase Conjugate (SP Conjugate)**

Spin down the SP Conjugate briefly and dilute the desired amount of the conjugate 1:100 with MIX Diluent to produce a 1X solution. The undiluted conjugate should be stored at -20°C.

9.4 **1X Biotinylated IGF1 Detector Antibody**

9.4.1 The stock Biotinylated Insulin like Growth Factor 1 Antibody must be diluted with 1X Diluent N according to the label concentration to prepare 1X Biotinylated Insulin like Growth Factor 1 Antibody for use in the assay procedure. Observe the label for the “X” concentration on the vial of Biotinylated Insulin like Growth Factor 1 Antibody.

9.4.2 Calculate the necessary amount of 1X Diluent N to dilute the Biotinylated Insulin like Growth Factor 1 Antibody to prepare a 1X Biotinylated Insulin like Growth Factor 1 Antibody solution for use in the assay procedure according to how many wells you wish to use and the following calculation:

Number of Wells Strips	Number of Wells	(V_T) Total Volume of 1X Biotinylated Antibody (µL)
4	32	1,760
6	48	2,640
8	64	3,520
10	80	4,400
12	96	5,280

Any undiluted solution should be stored at -20°C.

Where:

C_S = Starting concentration (X) of stock Biotinylated Insulin like Growth Factor 1 Antibody (variable)

C_F = Final concentration (always = 1X) of 1X Biotinylated Insulin like Growth Factor 1 Antibody solution for the assay procedure

V_T = Total required volume of 1X Biotinylated Insulin like Growth Factor 1 Antibody solution for the assay procedure

V_A = Total volume of (X) stock Biotinylated Insulin like Growth Factor 1 Antibody

V_D = Total volume of 1X Diluent N required to dilute (X) stock Biotinylated Insulin like Growth Factor 1 Antibody to prepare 1X Biotinylated Insulin like Growth Factor 1 solution for assay procedures

Calculate the volume of (X) stock Biotinylated Antibody required for the given number of desired wells:

$$(C_F / C_S) \times V_T = V_A$$

Calculate the final volume of 1X Diluent N required to prepare the 1X Biotinylated Insulin like Growth Factor 1 Antibody:

$$V_T - V_A = V_D$$

Example:

NOTE: This example is for demonstration purposes only. Please remember to check your antibody vial for the actual concentration of antibody provided.

C_S = 50X Biotinylated Insulin like Growth Factor 1 Antibody stock

C_F = 1X Biotinylated Insulin like Growth Factor 1 Antibody solution for use in the assay procedure

V_T = 3,520 µL (8 well strips or 64 wells)

$$(1X/50X) \times 3{,}520\ \mu L = 70.4\ \mu L$$

$$3{,}520\ \mu L - 70.4\ \mu L = 3{,}449.6\ \mu L$$

V_A = 70.4 µL total volume of (X) stock Biotinylated Insulin like Growth Factor 1 Antibody required

V_D = 3,449.6 µL total volume of 1X Diluent N required to dilute the 50X stock Biotinylated Antibody to prepare 1X Biotinylated Insulin like Growth Factor 1 Antibody solution for assay procedures

9.4.3 First spin the Biotinylated Insulin like Growth Factor 1 Antibody vial to collect the contents at the bottom.

9.4.4 Add calculated amount V_A of stock Biotinylated Insulin like Growth Factor 1 Antibody to the calculated amount V_D of 1X Assay Diluent N. Mix gently and thoroughly.

9.5 **1X SP Conjugate**

Spin down the 100X Streptavidin-Peroxidase Conjugate (SP Conjugate) briefly and dilute the desired amount of the conjugate 1:100 with 1X Diluent N.

Any remaining solution should be frozen at -20°C.

10. STANDARD PREPARATIONS

- **Prepare serially diluted standards immediately prior to use. Always prepare a fresh set of standards for every use.**
- **Any remaining standard should be stored at -20°C after reconstitution and used within 30 days.**
- **This procedure prepares sufficient standard dilutions for duplicate wells.**

10.1 Reconstitution of the Insulin like Growth Factor 1 Standard vial to prepare the 10 ng/mL Insulin like Growth Factor 1 **Standard #1.**

10.1.1 First consult the Insulin like Growth Factor 1 Standard vial to determine the mass of protein in the vial.

10.1.2 Calculate the appropriate volume of Standard Diluent to generate a 20 ng/mL stock solution by using the below equation.

10.1.3 Dilute 2-fold with 1X Diluent N to produce a 10 ng/mL Insulin like Growth Factor 1 **Standard #1**.

C_S = Starting mass of Insulin like Growth Factor 1 Standard (see vial label) (ng)

C_F = 20 ng/mL Insulin like Growth Factor 1 **Standard Stock** final required concentration

V_D = Required volume of Standard Diluent for reconstitution (µL)

Calculate total required volume Standard Diluent for resuspension:

$$(C_S / C_F) \times 1{,}000 = V_D$$

Example:

NOTE: This example is for demonstration purposes only. Please remember to check your standard vial for the actual amount of standard provided.

C_S = 10 ng of Insulin like Growth Factor 1 Standard in vial
C_F = 20 ng/mL Insulin like Growth Factor 1 **Standard Stock** final concentration
V_D = Required volume of 1X Diluent N for reconstitution

(10 ng / 20 ng/mL) x 1,000 = 500 µL

10.1.4 First briefly spin the Insulin like Growth Factor 1 Standard Vial to collect the contents on the bottom of the tube.

10.1.5 Reconstitute the Insulin like Growth Factor 1 Standard vial by adding the appropriate calculated amount V_D of Standard Diluent to the vial to generate the 20 ng/mL Insulin like Growth Factor 1 **Standard Stock**. Mix gently and thoroughly.

10.2 Allow the reconstituted 20 ng/mL Insulin like Growth Factor 1 **Standard Stock** to sit for 10 minutes with gentle agitation prior to making subsequent dilutions.

10.3 Label seven tubes #1 – 8.

10.4 To prepare **Standard #1**, dilute the stock standard 2-fold to produce a 10 ng/mL.

10.5 Add 120 µL of 1X Diluent N to tubes #2 – 8.

10.6 To prepare **Standard #2**, add 120 µL of the **Standard #1** into tube #2 and mix gently.

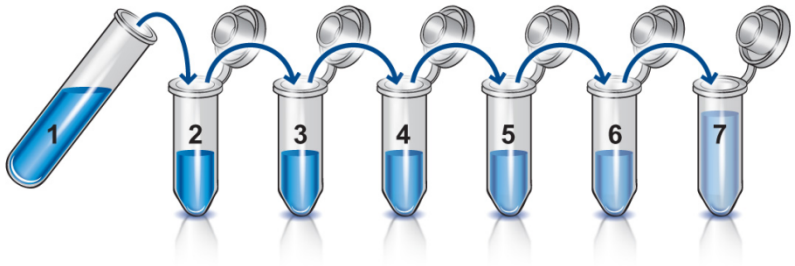
10.7 To prepare **Standard #3**, add 120 µL of the **Standard #2** into tube #3 and mix gently.

10.8 Using the table below as a guide, prepare subsequent serial dilutions.

10.9 1X Diluent N serves as the zero standard, 0 ng/mL (tube #8)

Standard Dilution Preparation Table

Standard #	Volume to Dilute (µL)	Volume Diluent N (µL)	Total Volume (µL)	Starting Conc. (ng/mL)	Final Conc. (ng/mL)
1	1 part standard stock (20 ng/mL) + 1 parts Diluent N				10.00
2	120	120	240	10.00	5.000
3	120	120	240	5.000	2.500
4	120	120	240	2.500	1.250
5	120	120	240	1.250	0.625
6	120	120	240	0.625	0.313
7	120	120	240	0.313	0.156
8	-	120	120	-	0



11. SAMPLE PREPARATION

11.1 Plasma

Collect plasma using one-tenth volume of 0.1 M sodium citrate as an anticoagulant. Centrifuge samples at 3,000 x *g* for 10 minutes. A 1:250 sample dilution is suggested into 1X Diluent N; 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.

11.2 Cell Culture Supernatant

Centrifuge cell culture media at 1500 x *g* for 10 minutes at 4°C to remove debris. Collect supernatant and assay. Samples can be stored at -80°C. Avoid repeated freeze-thaw cycles.

11.3 Serum

Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 3,000 x *g* for 10 minutes and remove serum. A 1:250 sample dilution is suggested into 1X Diluent N; 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.

Note: *Applicable samples may also include biofluids, cell culture, and tissue homogenates. If necessary, user should determine optimal dilution factor depending on application needs.*

12. PLATE PREPARATION

- The 96 well plate strips included with this kit are supplied ready to use. It is not necessary to rinse the plate prior to adding reagents.
- Unused well plate strips should be returned to the plate packet and stored at 4°C.
- For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates).
- Well effects have not been observed with this assay. Contents of each well can be recorded on the template sheet included in the Resources section.

13. ASSAY PROCEDURE

- **Equilibrate all materials and prepared reagents to room temperature (18 - 25°C) prior to use.**
- **It is recommended to assay all standards, controls and samples in duplicate.**

13.1 Prepare all reagents, working standards and samples as instructed. Equilibrate reagents to room temperature before use. The assay is performed at room temperature (18-25°C).

13.2 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.

13.3 Add 50 µL of Insulin like Growth Factor 1 standard or sample per 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 two hours. Start the timer after the last sample addition.

13.4 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.

13.5 Add 50 µL of 1X Biotinylated Insulin like Growth Factor 1 Antibody to each well and incubate for two hours.

13.6 Wash microplate as described above.

13.7 Add 50 µL of 1X 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.

13.8 Wash microplate as described above.

13.9 Add 50 µL of Chromogen Substrate per well. Gently tap plate to thoroughly coat the wells. Break any bubbles that may have formed. Incubate for about 20 minutes or till the optimal blue colour density develops.

13.10 Add 50 µL of Stop Solution to each well. Gently tap plate to thoroughly coat the wells. Break any bubbles that may have formed. The color will change from blue to yellow.

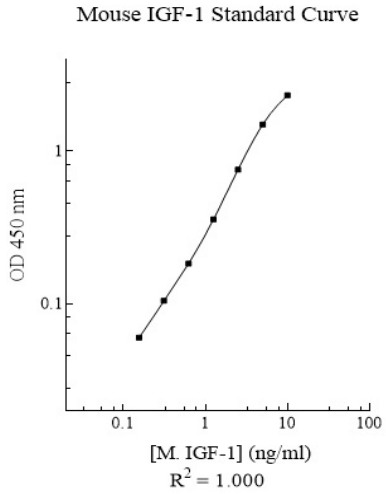
13.11 Read the absorbance on a microplate reader at a wavelength of 450 nm immediately. If wavelength correction is available, subtract readings at 570 nm from those at 450 nm to correct optical imperfections. Otherwise, read the plate at 450 nm only. Please note that some unstable black particles may be generated at high concentration points after stopping the reaction for about 10 minutes, which will reduce the readings.

14. CALCULATIONS

Calculate the mean value of the 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 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.

15. TYPICAL DATA

TYPICAL STANDARD CURVE – Data provided for demonstration purposes only. A new standard curve must be generated for each assay performed.



16. TYPICAL SAMPLE VALUES

SENSITIVITY –

The minimum detectable dose of mouse Insulin like Growth Factor 1 is typically~ 0.062 ng/mL.

RECOVERY –

Standard Added Value: 0.3 – 5 ng/mL

Recovery %: 87 – 112.

Average Recovery %: 96

LINEARITY OF DILUTION –

Plasma Dilution	Average % Expected Value
1:125	94
1:250	98
1:500	105

Serum Dilution	Average % Expected Value
1:125	96
1:250	101
1:500	104

PRECISION –

	Intra-Assay	Inter-Assay
% CV	3.2	9.8

17. ASSAY SPECIFICITY

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

10% FBS in culture media will not affect the assay.

18. TROUBLESHOOTING

Problem	Cause	Solution
Poor standard curve	Improper standard dilution	Confirm dilutions made correctly
	Standard improperly reconstituted (if applicable)	Briefly spin vial before opening; thoroughly resuspend powder (if applicable)
	Standard degraded	Store sample as recommended
	Curve doesn't fit scale	Try plotting using different scale
Low signal	Incubation time too short	Try overnight incubation at 4ºC
	Target present below detection limits of assay	Decrease dilution factor; concentrate samples
	Precipitate can form in wells upon substrate addition when concentration of target is too high	Increase dilution factor of sample
	Using incompatible sample type (e.g. serum vs. cell extract)	Detection may be reduced or absent in untested sample types
	Sample prepared incorrectly	Ensure proper sample preparation/dilution
Large CV	Bubbles in wells	Ensure no bubbles present prior to reading plate
	All wells not washed equally/thoroughly	Check that all ports of plate washer are unobstructed wash wells as recommended
	Incomplete reagent mixing	Ensure all reagents/master mixes are mixed thoroughly
	Inconsistent pipetting	Use calibrated pipettes and ensure accurate pipetting
	Inconsistent sample preparation or storage	Ensure consistent sample preparation and optimal sample storage conditions (eg. minimize freeze/thaws cycles)

Problem	Cause	Solution
High background/ Low sensitivity	Wells are insufficiently washed	Wash wells as per protocol recommendations
	Contaminated wash buffer	Make fresh wash buffer
	Waiting too long to read plate after adding STOP solution	Read plate immediately after adding STOP solution
	Improper storage of ELISA kit	Store all reagents as recommended. Please note all reagents may not have identical storage requirements.
	Using incompatible sample type (e.g. Serum vs. cell extract)	Detection may be reduced or absent in untested sample types

Technical Support

For all technical or commercial enquiries please go to:

www.abcam.com/contactus

www.abcam.cn/contactus (China)

www.abcam.co.jp/contactus (Japan)