



OCTEIA

Rat/Mouse IGF-I

Immunoenzymometric assay (IEMA) for the quantitative determination of Insulin-like Growth Factor I (IGF-I) in rat and mouse serum or plasma

Intended Use

For Research Use Only. Not for use in diagnostic procedures.

The OCTEIA Rat/Mouse IGF-I kit is a two-site immunoenzymometric assay [IEMA] for the quantitative determination of Insulin-like Growth Factor I in rat and mouse serum or plasma. The method incorporates a sample pre-treatment to avoid interference from binding proteins.

Summary and Explanation

Insulin-like growth factor I (IGF-I) plays a major role in mammalian growth and regenerative processes, and mediates the actions of growth hormone (GH) ^[1]; therefore measurement is of considerable interest in human diagnostics and in animal research. IGF-I is a 70 amino acid peptide (7.6kDa), and is one of a number of related insulin-like growth factors present in the circulation. In vivo, approximately 99% of the IGFs in the circulation are associated with IGF binding proteins (IGF-BP 1-6) ^[2,3].

The mature peptide is highly conserved between species and is also structurally similar to IGF-II and insulin. However, rat and mouse IGF-I does differ from human IGF-I. Three scattered amino acid substitutions (position 20, 35 and 67) distinguish rat from human IGF-I and mouse IGF-I differs by a further substitution (position 69). Even though there are only a small number of substitutions, most 2-site human IGF-I assays are not able to measure rat or mouse IGF-I.

Method Description

Rat and mouse samples are incubated briefly with a reagent to inactivate binding proteins, and then diluted for assay. In the OCTEIA Rat / Mouse IGF-I kit, a purified monoclonal anti-Rat IGF-I is coated onto the inner surface of polystyrene microtitre wells (the solid phase or capture antibody). The pre-treated, diluted sample is then incubated, together with biotinylated polyclonal rabbit anti-rat IGF-I, in antibody coated wells and shaken for 2 hours at room temperature. The wells are washed and enzyme (horseradish peroxidase) labelled avidin is added which binds to the biotin complex. After a further wash, a single component chromogenic substrate (a formulation of tetramethyl-benzidine) is added to develop colour. The absorbance of the stopped reaction mixture is read in a microtitre plate reader, colour intensity developed being directly proportional to the amount of rat and mouse IGF-I present in the sample.

Warnings and Precautions

The OCTEIA Rat / Mouse IGF-I kit is for research use only and is not for internal use in humans or animals. This product must be used strictly in accordance with the instructions set out in the Package Insert. IDS Limited will not be held responsible for any loss or damage (except as required by statute) howsoever caused, arising out of non-compliance with the instructions provided.

CAUTION: this kit contains material of human and/or animal origin. Handle kit reagents as if capable of transmitting an infectious agent.

Appropriate precautions and good laboratory practices must be used in the storage, handling and disposal of the kit reagents. Disposal of kit reagents should be in accordance with local regulations.

Human Serum: Antibody Biotin AB BIOTIN

Human material used in the preparation of this product has been tested by FDA recommended assays and found to be non-reactive for the presence of antibody to Human Immunodeficiency Virus (HIV I and II), Hepatitis B surface antigen and antibody to Hepatitis C. As no test can offer complete assurance that infectious agents are absent, the reagents should be handled in accordance at Biosafety Level 2.

Sodium Azide

Some reagents in this kit contain sodium azide as a preservative, which may react with lead, copper or brass plumbing to form highly explosive metal azides. On disposal, flush with large volumes of water to prevent azide build up.

Tetramethylbenzidine

TMB Substrate TMB contains 3,3',5,5'-tetramethylbenzidine.

R21/22 Harmful by contact with skin and if swallowed.

S36/37 Wear suitable protective clothing and gloves.

0.5M hydrochloric acid

Stop Solution HCL contains 0.5M hydrochloric acid.

R36/38 Irritating to eyes and skin.

S26 In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.

S36/37 Wear suitable protective clothing and gloves.

Preparation of Reagents

Calibrators [CAL] and Controls [CTRL]: Calibrators [CAL] and Controls [CTRL] are supplied lyophilised. Reconstitute with 0.5 mL of distilled or deionised water, replace stopper and stand for 5 to 10 minutes at room temperature. Invert several times to ensure complete reconstitution.

If Calibrators [CAL] or Controls [CTRL] are to be used more than once, they must be frozen (-20°C) promptly (<30 minutes post-reconstitution).

When re-using frozen Calibrators [CAL] and Controls [CTRL], thaw at room temperature, with mixing, and use within 15 minutes of completion of thaw.

Wash Solution: Add the contents of each bottle of Wash Concentrate [WASHBUF] [20x] to 950 mL of distilled or de-ionised water and mix. Store at room temperature.

All other reagents are supplied ready for use.

Allow all reagents to come to room temperature before use. Reagents should be mixed by repeated inversion prior to use in the assay.

Shelf Life and Storage of Reagents

This kit is stable until the stated expiry date if stored as specified. Upon receipt, store all reagents at 2-8°C.

Reconstituted Calibrators [CAL] and Controls [CTRL] can be stored at -20°C for up to 8 weeks.

Indications of possible deterioration of kit reagents.

1. The presence of abnormal particulate matter in any of the reagents.
2. A decrease in the maximum absorbance.
3. A shift in the slope of the curve from its normal position.

Specimen Collection and Storage

The assay should be performed using either serum or plasma specimens. Specimens should be separated as soon as possible after collection. For long term storage, store at -20°C. Avoid repeated freeze/thaw of samples

Note: Improper handling and storage of samples may result in loss of assayable rat/mouse IGF-I.

Procedure

Materials Provided

1. CAL 0 - 5 - Calibrators (REF AC-1801A - AC-1801F):

Lyophilised phosphate buffer containing rat IGF-I and 0.05% sodium azide. The exact value of each Calibrator is printed on the bottle label, 0.5 mL per bottle, 6 bottles per kit.

2. MICROPLAT - Antibody Coated Plate (REF AC-1802W):

Microplate with anti-rat IGF-I monoclonal antibody linked to the inner surface of the polystyrene wells, 12 x 8 well strips in a foil pouch with desiccant.

3. AB BIOTIN - Anti-rat IGF-I Biotin (REF AC-1803):

Phosphate buffered saline containing rabbit anti-rat IGF-I polyclonal antibody labelled with biotin, protein, proprietary stabilisers and preservative. 12 mL per bottle.

4. ENZYMCNJ - Enzyme Conjugate (REF AC-1804):

Phosphate buffered saline containing avidin linked to horseradish peroxidase, protein, enzyme stabilisers and preservative, 24 mL per bottle.

5. CTRL 1 - 2 - Controls (REF AC-1805A - AC-1805B):

Lyophilised rat serum, 0.5 mL per bottle, 2 bottles per kit.

6. HCL - Stop Solution (REF AC-STOP):

0.5M hydrochloric acid, 14 mL per bottle.

7. RELEASREAG - Releasing Reagent (REF AC-1807):

Proprietary reagent for dissociating IGF-I from binding proteins, 6 mL per bottle.

8. TMB - TMB Substrate (REF AC-TMB):

A proprietary aqueous formulation of tetramethylbenzidine (TMB) and hydrogen peroxide, 24 mL per bottle.

9. SAMPDIL - Sample Diluent (REF AC-1809):

Phosphate-buffered saline containing protein and 0.05% sodium azide, 50 mL per bottle.

10. WASHBUF 20x - Wash Concentrate (REF AC-WASHL):

Phosphate buffered saline containing Tween, 50 mL per bottle.

11. Adhesive plate sealer

4 per kit.

12. Documentation - Package Insert, QC Report, Symbol Explanation Sheet

Materials Required but not Provided

1. Disposable 12 x 75 mm plastic or glass tubes.
2. Precision pipetting devices to deliver 25 μL .
3. Repeating pipettes to deliver 100 μL and 1 mL, e.g. Eppendorf Multipipette 4780, or similar
4. Precision multi-channel pipettes to deliver 100 μL and 200 μL .
5. Vortex mixer.
6. Automatic microplate washer (optional).
7. Photometric microplate reader and data analysis equipment.
8. Orbital plate shaker.

Sample Pre-treatment

Reconstitute or prepare reagents as described in "Preparation of Reagents".

1. Prepare labelled plastic or glass tubes, one for each Control **CTRL** and sample.
*Note: Calibrators **CAL** DO NOT REQUIRE pre-treatment.*
2. Add **25 μL** of each Control **CTRL** or sample to appropriately labelled tubes.
3. Add **100 μL** of Releasing Reagent **RELEASREAG** to each tube. Vortex all tubes. Incubate at 18-28°C for 10 minutes.
4. Add **1.0 mL** of Sample Diluent **SAMPDIL** to each tube. Vortex all tubes. Controls and samples are now ready to assay.

Alternative sample pre-treatment protocol for smaller sample volumes, <25 μL :

1. Prepare labelled plastic or glass tubes, one for each sample.
2. Add each sample (e.g. **10 μL**) to appropriately labelled tubes.
3. Add 4 x sample volume (e.g. **40 μL**) of Releasing Reagent **RELEASREAG** to each tube. Vortex all tubes. Incubate at 18-28°C for 10 minutes.
4. Add 40 x sample volume (e.g. **400 μL**) of Sample Diluent **SAMPDIL** to each tube. Vortex all tubes. Samples are now ready to assay.

Assay Procedure

1. Add **25 µL** of each Calibrator [CAL], or diluted Control or diluted sample to the appropriate wells of the Antibody Coated Plate [MICROPLAT] in duplicate.

Note: these should be dispensed within a period of 30 minutes to minimise assay drift.

2. Add **100 µL** of Anti-rat IGF-I Biotin [AB BIOTIN] to all wells using a multichannel pipette.
3. Cover the plate with an adhesive plate sealer and incubate the plate on an orbital microplate shaker (500-700rpm) at 18-28°C for 2 hours.
4. Wash all wells three times with Wash Solution:
 - a. Automatic plate wash: Set plate washer to dispense at least **300 µL** of Wash Solution per well. Fill and aspirate for 3 cycles.
 - b. Manual wash: Decant the contents of the wells by inverting sharply. Dispense **250 µL** of Wash Solution to all wells. Decant and repeat twice.

Tap the inverted plate firmly on absorbent tissue to remove excess Wash Solution before proceeding to the next step.

5. Add **200 µL** of Enzyme Conjugate [ENZYMCONJ] to all wells using a multichannel pipette. Incubate at 18-28°C for 30 minutes.
6. Repeat wash step 4.
7. Add **200 µL** of TMB Substrate [TMB] to all wells using a multichannel pipette. Incubate at 18-28°C for 30 minutes.

Note: TMB Substrate is easily contaminated. Only remove the required amount for the assay from the bottle. Dispose of unused TMB Substrate. Do not return to bottle.

8. Add **100 µL** of Stop Solution [HCL] to all wells using a multichannel pipette.
9. Measure the absorbance of each well at 450 nm (reference 650 nm) using a microplate reader within 30 minutes of adding the Stop Solution.

Quality Control

The regular use of control samples at several analyte levels is advised to ensure day-to-day validity of results. Two kit controls are provided. The controls should be tested as unknowns. Quality Control charts should be maintained to follow the assay performance.

Calculation of Results

Calculate the mean absorbance for each Calibrator, Control and unknown sample. Prepare a calibration curve on lin-log graph paper by plotting the mean absorbance for each Calibrator on the ordinate against concentration of rat IGF-I on the abscissa. Read values for each control and unknown sample from the calibration curve in ng/mL. For the estimation of low values we recommend a plot of absorbance against concentration of low calibrator values on linear-linear graph paper. Results for unknown samples can be read directly from the curve.

Alternative data reduction techniques may be employed but users should confirm that the selected curve fit is appropriate and gives acceptable results. 4PL curve fits are recommended.

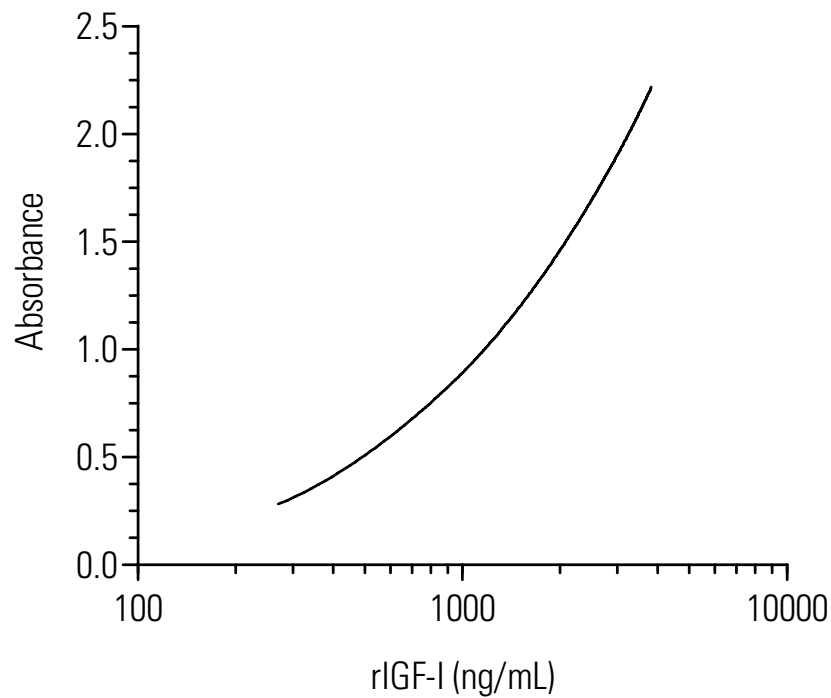
Sample Assay Data

This data is for illustration only and must not be used for the calculation of any sample result.

Well	Description	Duplicate Absorbance	Mean Absorbance	Result (ng/mL)
A1, A2	Calibrator 0 (0 ng/mL)	0.142 0.156	0.149	
B1, B2	Calibrator 1 (271 ng/mL)	0.283 0.280	0.282	
C1, C2	Calibrator 2 (492 ng/mL)	0.495 0.507	0.501	
D1, D2	Calibrator 3 (1031 ng/mL)	0.915 0.909	0.912	
E1, E2	Calibrator 4 (2086 ng/mL)	1.508 1.497	1.503	
F1, F2	Calibrator 5 (3821 ng/mL)	2.229 2.205	2.217	
G1, G2	Sample 1	0.577 0.561	0.569	598
H1, H2	Sample 2	1.125 1.211	1.168	1457

Typical Calibration Curve

This sample calibration curve is for illustration only.



Limitations of Use

1. Samples suspected of containing analyte concentrations in excess of the highest calibrator should be diluted in Calibrator 0 and re-assayed.
2. The following substances have been tested and found not to interfere in the OCTEIA Rat/Mouse IGF-I assay:
 - Haemoglobin tested up to 500 mg/dL
 - Bilirubin tested up to 400 $\mu\text{g/mL}$
 - Lipid tested up to 4000 mg/dL triglyceride
 - rIGF-BP4 tested up to 8000 ng/mL
 - rIGF-II tested up to 2000 ng/mL
4. The hook effect was tested using concentrations of rat IGF-I up to 20,000 ng/mL. No hook effect was observed.

Performance Data

Sensitivity

The sensitivity, defined as the concentration corresponding to the mean plus 2 standard deviations of 20 replicates of the zero Calibrator, is 63 ng/mL.

Precision

Intra-Assay Variation (n=20)		Inter-Assay Variation (n=26)	
Mean Value (ng/mL)	%CV	Mean Value (ng/mL)	%CV
412	7.3	393	8.8
923	8.8	905	6.8
1593	4.3	1544	6.3

Linearity

Linearity was assessed by diluting high samples with Calibrator 0 prior to assay.

Sample	Measured ng/mL	Expected ng/mL	% M/E
Low (Cal 0)	-	-	-
0.75L + 0.25H	453	420	108
0.50L + 0.50H	946	839	113
0.25L + 0.75H	1275	1259	101
High	1678	-	-
Low (Cal 0)	-	-	-
0.75L + 0.25H	369	320	115
0.50L + 0.50H	661	639	103
0.25L + 0.75H	873	959	91
High	1278	-	-
		Mean	105

Recovery

Recovery was assessed by adding rat IGF-I to samples prior to assay.

Sample conc. ng/mL	IGF-I added ng/mL	Measured ng/mL	Recovery ng/mL	Recovery %
1579	400	1946	367	91
1579	800	2248	669	84
1485	400	1817	332	83
1485	800	2178	693	87
			Mean	86

Specificity

The specificity of the OCTEIA Rat / Mouse IGF-I has been assessed with the following analytes:

Analyte	Cross-Reactivity
Rat IGF-I	100 %
Mouse IGF-I	100 %
Rat IGF-II	Not Detectable
Human IGF-I	Not Detectable

References

1. Daughaday E, Rotwein, P. *Insulin like growth factor I and II. Peptide, messenger ribonucleic acid and gene structure, serum and tissue concentrations. Endocrin Rev 1989: 10: 68-91.*
2. Baxter, RC, Martin JL, Beniac VA. *High molecular weight insulin-like growth factor binding protein complex. J Biol Chem 1989: 264: 11843-11848.*
3. Rechler M. *Insulin-like growth factor binding proteins. Vit Horm 1993: 47: 1-114.*

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Procedure Summary

SAMPLE PRE-TREATMENT

25 μ L Control [CTRL] or sample.

Add 100 μ L Releasing Reagent [RELEASREAG]. Vortex.



Incubate: 10 minutes @ 18-28°C.



Add 1mL Sample Diluent [SAMPDIL]. Vortex.

ASSAY

Add 25 μ L Calibrator [CAL], diluted Control or diluted sample to

Antibody Coated Plate [MICROPLAT].

Add 100 μ L Anti-Rat IGF-I Biotin [AB BIOTIN].



Incubate: shaken 2 hours @ 18-28°C



Wash plate.



Add 200 μ L Enzyme Conjugate [ENZYMCONJ].



Incubate: 30 minutes @ 18-28°C.



Wash plate.



Add 200 μ L TMB Substrate [TMB].



Incubate: 30 minutes @ 18-28°C.



Add 100 μ L Stop Solution [HCL].



Read plate @ 450nm (reference 650nm).

Developed in association with:

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