Hamster IGF-1 ELISA Kit

Cat#: orb546436 (ELISA Manual)

Assay Principle

The Biorbyt Hamster Igf1 Pre-Coated ELISA (Enzyme-Linked Immunosorbent Assay) kit is a solid-phase immunoassay specially designed to measure Hamster Igf1 with a 96-well strip plate that is pre-coated with antibody specific for Igf1. The detection antibody is a biotinylated antibody specific for Igf1. The capture antibody is monoclonal antibody from mouse, the detection antibody is polyclonal antibody from goat specific for IGF-1. The kit contains recombinant Hamster Igf1 with immunogen: Expression system for standard: E.coli; Immunogen sequence: G49-A118. The kit is analytically validated with ready-to-use reagents. To measure Hamster Igf1, add standards and samples to the wells, then add the biotinylated detection antibody. Wash the wells with PBS or TBS buffer, and add Avidin-Biotin-Peroxidase Complex (ABC-HRP). Wash away the unbounded ABC-HRP with PBS or TBS buffer and add TMB. TMB is an HRP substrate and will be catalyzed to produce a blue color product, which changes into yellow after adding the acidic stop solution. The density of the yellow product is linearly proportional to Hamster Igf1 in the sample. Read the density of the yellow product in each well using a plate reader, and benchmark the sample wells' readings against the standard curve to determine the concentration of Hamster Igf1 in the sample.

Overview

Product Name Chinese Hamster IGF-1 ELISA Kit
Reactive Species Hamster
Size 96 wells/kit, with removable strips.
Description Chinese Hamster IGF-1 ELISA Kit (96 Tests). Quantitate Chinese Igf1 in cell culture supernatants, serum and plasma (heparin, EDTA). Sensitivity: 5pg/ml.
Sensitivity <5 pg/ml
*The sensitivity or the minimum detectable dose (MDD) is the lower limit of the target protein that can be detected by the kit. It is determined by adding two standard deviations to the mean O.D. value of twenty (20) blank wells and calculating the corresponding concentration.
Detection Range 62.5 pg/ml - 4,000 pg/ml
Storage Instructions Store at 4˚C for 6 months, at -20°C for 12 months. Avoid multiple freeze-thaw cycles (Shipped with wet ice.)
Uniprot ID P08025

Technical Details

Capture/Detection Antibodies
The capture antibody is monoclonal antibody from mouse, the detection antibody is polyclonal antibody from goat specific for IGF-1.

Specificity
Natural and recombinant Hamster Igf1

Immunogen
Expression system for standard: E.coli; Immunogen sequence: G49-A118

Cross Reactivity
This kit is for the detection of Hamster Igf1. No significant cross-reactivity or interference between Igf1 and its analogs was observed. This claim is limited by existing techniques therefore cross-reactivity may exist with untested analogs.

Preparations Before Assay

Please read the following instructions before starting the experiment.
1. Read this manual in its entirety in order to minimize the chance of error.
2. Confirm that you have the appropriate non-supplied equipment available.
3. Confirm that the species, target antigen, and sensitivity of this kit are appropriate for your intended application.
4. Confirm that your samples have been prepared appropriately based upon recommendations (see Sample Preparation) and that you have sufficient sample volume for use in the assay.
5. When first using a kit, appropriate validation steps should be taken before using valuable samples. Confirm that the kit adequately detects the target antigen in your intended sample type(s) by running control samples.
6. If the concentration of target antigen within your samples is unknown, a preliminary experiment should be run using a control sample to determine the optimal sample dilution (see Sample Preparation).
7. To inspect the validity of experiment operation and the appropriateness of sample dilution proportion, a pilot experiment using standards and a small number of samples is recommended.
8. Before using the kit, spin tubes to bring down all components to the bottom of the tubes.
9. Don't let the 96-well plate dry out since this will inactivate active components on the plate.
10. Don't reuse tips and tubes to avoid-cross contamination.
11. Avoid using the reagents from different batches together.
12. The kit should not be used beyond the expiration date on the kit label. Any variation in diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding. Variations in sample collection, processing, and storage may cause sample value differences.

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Kit Components/Materials Provided

Description	Quantity	Volume	Storage of opened/reconstituted material
Anti-Hamster Igf1 Pre-coated 96-well Strip Microplate	1	12 strips of 8 wells	Return unused wells to the foil pouch. Reseal along the entire edge of the zip-seal. May be stored for up to 1 month at 4°C provided this is within the expiration date of the kit.
Hamster Igf1 Standard	2	10 ng/tube	Discard the Igf1 stock solution after 12 hours at 4°C. May be stored at -20°C for 48 hours.
Hamster Igf1 Biotinylated Antibody (100x)	1	100 µl	May be stored for up to 1 month at 4°C provided this is within the expiration date of the kit.
Avidin-Biotin-Peroxidase Complex (100x)	1	100 µl	
Sample Diluent	1	30 ml	
Antibody Diluent	1	12 ml	
Avidin-Biotin-Peroxidase Diluent	1	12 ml	
Color Developing Reagent (TMB)	1	10 ml	
Stop Solution	1	10 ml	
Wash Buffer (25x)	1	20 ml	
Plate Sealers	4	Piece	

Required Materials That Are Not Supplied

Microplate reader capable of reading absorbance at 450nm.
Automated plate washer (optional)
Pipettes and pipette tips capable of precisely dispensing 0.5 µl through 1 ml volumes of aqueous solutions.
Multichannel pipettes are recommended for a large number of samples.
Deionized or distilled water.
500ml graduated cylinders.
Test tubes for dilution.

Chinese Hamster IGF-1 ELISA Kit Standard Curve Example

The highest O.D. value might be higher or lower than in the example. The experiment result is statistically significant if the highest O.D. value is no less than 1.0.

Concentration (pg/ml)	0	62.5	125	250	500	1000	2000	4000
O.D.	0.023	0.080	0.141	0.258	0.609	1.233	1.634	2.085

Hamster IGF-1 ELISA Kit standard curve
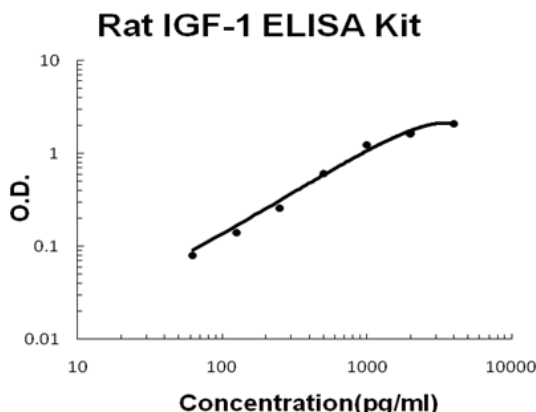
A standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.

Intra/Inter Assay Variability
Biorbyt spends great efforts in documenting lot-to-lot variability and ensuring our assay kits produce robust data that are reproducible.

Intra-Assay Precision (Precision within an assay): Three samples of known concentration were tested on one plate to assess intra-assay precision.

Inter-Assay Precision (Precision across assays): Three samples of known concentration were tested in separate assays to assess interassay precision.

Sample	Intra-Assay Precision			Inter-Assay Precision		
	1	2	3	1	2	3
n	16	16	16	24	24	24
Mean(pg/ml)	527	1441	2641	497	1532	2737
Standard deviation	32.67	56.20	140	41.25	82.73	156
CV(%)	6.2	3.9	5.3	8.3	5.4	5.7

Preparation Before The Experiment

All reagents
Bring all reagents to room temperature (18-25°C) prior to use. Please DO NOT equilibrate unused plate well strips to room temperature. They should be sealed and stored in the original packaging. We recommend doing it at 37°C for best consistency with our QC results. Also, the TMB incubation time estimate (15-25 min) is based on incubation at 37°C.

Wash buffer
Prepare 500 ml of Working Wash Buffer by diluting the supplied 20 ml of Wash Buffer (25 x) with 480 ml of deionized or distilled water. If crystals have formed in the concentrate, warm to room temperature and mix it gently until crystals have completely dissolved.

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Biotinylated Anti-Hamster Igf1 antibody
It is recommended to prepare this reagent immediately prior to use by diluting the Hamster Igf1 Biotinylated antibody (100x) 1:100 with Antibody Diluent. Prepare 100 µl by adding 1 µl of Biotinylated antibody (100x) to 99 µl of Antibody Diluent for each well. Mix gently and thoroughly and use within 2 hours of generation.

Avidin-Biotin-Peroxidase Complex
It is recommended to prepare this reagent immediately prior to use by diluting the Avidin-Biotin-Peroxidase Complex (100x) 1:100 with Avidin-Biotin-Peroxidase Diluent. Prepare 100 µl by adding 1 µl of Avidin-Biotin-Peroxidase Complex (100x) to 99 µl of Avidin-Biotin-Peroxidase Diluent for each well. Mix gently and thoroughly and use within 2 hours of generation.

Hamster Igf1 Standard
It is recommended that the standards be prepared no more than 2 hours prior to performing the experiment. Use one 10 ng of lyophilized Hamster Igf1 standard for each experiment. Gently spin the vial prior to use. Reconstitute the standard to a stock concentration of 10 ng/ml using 1ml of sample diluent. Allow the standard to sit for a minimum of 10 minutes with gentle agitation prior to making dilutions.

Microplate
The included microplate is coated with capture antibodies and is ready-to-use. It does not require additional washing or blocking. The unused well strips should be sealed and stored in the original packaging.

Dilution of Hamster Igf1 Standard

1. Number tubes 1-8. Final Concentrations to be Tube # 1 –4000pg/ml, #2 –2000pg/ml, #3 – 1000pg/ml, #4 – 500pg/ml, #5 – 250pg/ml, #6 – 125pg/ml, #7 – 62.5pg/ml, #8 – Sample Diluent serves as the zero standard (0pg/ml).
2. To generate standard #1, add 400µl of the reconstituted standard stock solution of 10 ng/ml and 600µl of sample diluent to tube #1 for a final volume of 1000µl. Mix thoroughly.
3. Add 300 µl of sample diluent to tubes # 2-7.
4. To generate standard #2, add 300 µl of standard #1 from tube #1 to tube #2 for a final volume of 600 µl. Mix thoroughly.
5. To generate standard #3, add 300 µl of standard #2 from tube #2 to tube #3 for a final volume of 600 µl. Mix thoroughly.
6. Continue the serial dilution for tube #4-7.

Sample Preparation and Storage

These sample collection instructions and storage conditions are intended as a general guideline, and the sample stability has not been evaluated.

Cell culture supernatants
Clear sample of particulates by centrifugation, assay immediately or store samples at -20°C.

Serum

Use a serum separator tube (SST) and allow serum to clot at room temperature for about four hours. Then, centrifuge for 15 min at approximately 1,000 x g. assay immediately or store samples at -20°C.

Plasma

Collect plasma using heparin or EDTA as an anticoagulant. Centrifuge for 15 min at approximately 1,000 x g. Assay immediately or store samples at -20°C.

*Note: it is important to not use anticoagulants other than the ones described above to treat plasma for other anticoagulants could block the antibody binding site.

Sample Collection Notes

1. Biorbyt recommends that samples are used immediately upon preparation.
2. Avoid repeated freeze/thaw cycles for all samples.
3. In the event that a sample type not listed above is intended to be used with the kit, it is recommended that the customer conduct validation experiments in order to be confident in the results.
4. Due to chemical interference, the use of tissue or cell extraction samples prepared by chemical lysis buffers may result in inaccurate results.
5. Due to factors including cell viability, cell number, or sampling time, samples from cell culture supernatant may not be detected by the kit.
6. Samples should be brought to room temperature (18-25°C) before performing the assay without the use of extra heating.
7. Sample concentrations should be predicted before being used in the assay. If the sample concentration is not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.
8. Biorbyt is responsible for the quality and performance of the kit components but is NOT responsible for the performance of customer supplied samples used with the kit.

Sample Dilution Guideline

The user needs to estimate the concentration of the target protein in the sample and use an appropriate dilution factor so that the diluted target protein concentration falls in the range of O.D. values of the standard curve. Dilute the sample using provided diluent buffer. Pilot tests using a dilution series of each sample type are necessary. The sample must be mixed thoroughly with Sample Diluent.

Assay Protocol

It is recommended that all reagents and materials be equilibrated to room temperature (18-25°C) prior to the experiment (see Preparation Before The Experiment, if you have missed this information).
1. Prepare all reagents and working standards as directed previously.
2. Remove excess microplate strips from the plate frame and seal and store them in the original packaging.
3. Add 100 µl of the standard, samples, or control per well. Add 100 µl of the **Sample Diluent** into the zero well. At least two replicates of each standard, sample, or control is recommended.

4. Cover with the plate sealer provided and incubate for 120 minutes at room temperature (or 90 min. at 37 °C).

5. Remove the cover and discard the liquid in the wells into an appropriate waste receptacle. Invert the plate on the benchtop onto a paper towel and tap the plate to gently blot any remaining liquid. It is recommended that the wells are not allowed to completely dry at any time.

6. Add 100 µl of the prepared **1x Biotinylated Anti-Hamster Igf1 antibody** to each well.

7. Cover with a plate sealer and incubate for 90 minutes at room temperature (or 60 minutes at 37°C).

8. Wash the plate 3 times with the **1x wash buffer**:

a. Discard the liquid in the wells into an appropriate waste receptacle. Then, invert the plate on the benchtop onto a paper towel and tap the plate to gently blot any remaining liquid. It is recommended that the wells are not allowed to completely dry at any time.

b. Add 300 µl of the **1x wash buffer** to each assay well. (For cleaner background incubate for 60 seconds between each wash).

c. Repeat steps a-b 2 additional times.

9. Add 100 µl of the prepared **1x Avidin-Biotin-Peroxidase Complex** into each well. Cover with the plate sealer provided and incubate for 40 minutes at RT (or 30 minutes at 37°C).

10. Wash the plate 5 times with the **1x wash buffer**:

a. Discard the liquid in the wells into an appropriate waste receptacle. Then, invert the plate on the benchtop onto a paper towel and tap the plate to gently blot any remaining liquid. It is recommended that the wells are not allowed to completely dry at any time.

b. Add 300 µl of the **1x wash buffer** to each assay well. (For cleaner background incubate for 60 seconds between each wash).

c. Repeat steps a-b 4 additional times.

11. Add 90 µl of **Color Developing Reagent** to each well. Cover with the plate sealer provided and incubate in the dark for 30 minutes at RT (or 15-25 minutes at 37°C). (The optimal incubation time must be empirically determined. A guideline to look for is blue shading the top four standard wells, while the remaining standards remain clear.)

12. Add 100 µl of **Stop Solution** to each well. The color should immediately change to yellow.

13. Within 30 minutes of stopping the reaction, the O.D. absorbance should be read with a microplate reader at 450nm.

Assay Protocol Notes

1. Solutions: To avoid cross-contamination, change pipette tips between additions of each standard, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.

2. Applying Solutions: All solutions should be added to the bottom of the ELISA plate well. Avoid touching the inside wall of the well. Avoid foaming when possible.

3. Assay Timing: The interval between adding samples to the first and last wells should be minimized. Delays will increase the incubation time differential between wells, which will significantly affect the experimental accuracy and repeatability. For each step in the procedure, total dispensing time for addition of reagents or samples should not exceed 10 minutes.

4. Incubation: To prevent evaporation and ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary. Do not allow wells to sit uncovered for extended periods of time between

incubation steps. Do not let wells dry out at any time during the assay. Strictly observe the recommended incubation times and temperatures.

5. Washing: Proper washing procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings. Residual liquid in the reaction wells should be patted dry against absorbent paper during the washing process. Do not put absorbent paper directly into the reaction wells.

6. Controlling Substrate Reaction Time: After the addition of the TMB Substrate, periodically monitor the color development. Stop color development before the color becomes too deep by adding Stop Solution. The excessively strong color will result in inaccurate absorbance readings.

7. Reading: The microplate reader should be preheated and programmed prior to use. Prior to taking OD readings, remove any residual liquid or fingerprints from the underside of the plate and confirm that there are no bubbles in the wells.

8. Reaction Time Control: Control reaction time should be strictly followed as outlined.

9. Stop Solution: The Stop Solution contains an acid, therefore proper precautions should be taken during its use, such as protection of the eyes, hands, face, and clothing.

10. To minimize the external influence on the assay performance, operational procedures and lab conditions (such as room temperature, humidity, incubator temperature) should be strictly controlled. It is also strongly suggested that the whole assay is performed by the same operator from the beginning to the end.

Data Analysis

Average the duplicate readings for each standard, sample, and control. Subtract the average zero standard O.D. reading. It is recommended that a standard curve be created using computer software to generate a four-parameter logistic (4-PL) curve-fit. Alternatively, plot the mean absorbance for each standard against the concentration. The measured concentration in the sample can be interpolated by using linear regression of each average relative OD against the standard curve generated using curve fitting software. This will generate an adequate but less precise fit of the data. For diluted samples, the concentration reading from the standard curve must be multiplied by the dilution factor.

Background on Igf1

Insulin-like growth factor 1 (IGF-1) that was once called somatomedin C, is a polypeptide protein hormone similar in molecular structure to insulin. It plays an important role in childhood growth and continues to have anabolic effects in adults. Chinese hamster IGF1 is a single chain 70-amino acid polypeptide cross-linked by 3 disulfide bridges, with a calculated molecular mass of 7.6 kD. The IGF1 gene, mapped on 12q22-q24.1, contains 5 exons. Exons 1-4 encode the 195-amino acid precursor (IGF1B), and exons 1, 2, 3, and 5 encode the 153-residue peptide (IGF1A). The structure of IGF1 resembles that of IGF2. And the IGF1 and IGF2 genes have complex structures with multiple promoters. The expression of both genes is regulated at the levels of transcription, RNA processing, and translation. IGF-1 is produced primarily by the liver as an endocrine hormone as well as in target tissues in a paracrine/autocrine fashion. Moreover, approximately 98% of IGF-1 is always bound to one of 6 binding proteins (IGF-BP). Furthermore, IGF-1 is one of the most potent natural activators of the AKT signaling pathway, a stimulator of cell growth and multiplication and a potent inhibitor of programmed cell death.