PRODUCT INFORMATION & ELISA MANUAL

IGF-I R/IGF1R Antibody Pair [Biotin]

NBP2-79459

Sample Insert for reference use only

Matched Antibody Pair utilized in an Enzyme-linked Immunosorbent Assay for quantitative detection of Human IGF-I R/IGF1R.

For research use only.

Not for diagnostic or therapeutic procedures.

www.novusbio.com - P: 303.730.1950 - P: 888.506.6887 - F: 303.730.1966 - technical@novusbio.com

BACKGROUND

Insulin-like growth factor 1 receptor, also known as Insulin-like growth factor I receptor , IGF1R, and CD221, is a single-pass type I membrane protein which belongs to the protein kinase superfamily, Tyr protein kinase family and Insulin receptor subfamily. It is a disulfide-linked heterotetrameric transmembrane protein consisting of two α and two β subunits, and among which, the α subunit is extracellular while the β subunit has an extracellular domain, a transmembrane domain and a cytoplasmic tyrosine kinase domain. IGF1 receptor is a receptor tyrosine kinase (RTK) expressed in all cell types and tissues, and plays a critical role in transformation events during embryogenesis, development and pathogenesis processes. IGF1R become tyrosine phosphorylated and enzymatically activated either in response to IGF1 and 2 ligands or because of the activity of the Src tyrosine kinase, and subsequently initiates an intracellular signaling cascade (MAPK) that leads to gene expression changes and correspondent cellular responses.

IGF1R is commonly overexpressed in most malignant tissues including breast, prostate, pancreas and affect cancer cell proliferation, adhesion, metastasis and survival by mediating the anti-apoptotic pathway. IGF1R is regarded as potential anti-tumor therapeutic target, and momoclonal antibodies are probably the most promising and specific therapeutic agents. Defects in IGF1R may be a cause in some cases of resistance to insulin-like growth factor 1 (IGF1 resistance). IGF1 resistance is a gowth deficiency disorder characterized by intrauterine growth retardation and poor postnatal growth accompanied with increased plasma IGF1. Specificity for IGF1R targeting can be achieved by antisense and siRNA-mediated IGF1R downregulation; these approaches have undoubted utility as research tools, and may in future generate nucleic-acid-based therapeutics.

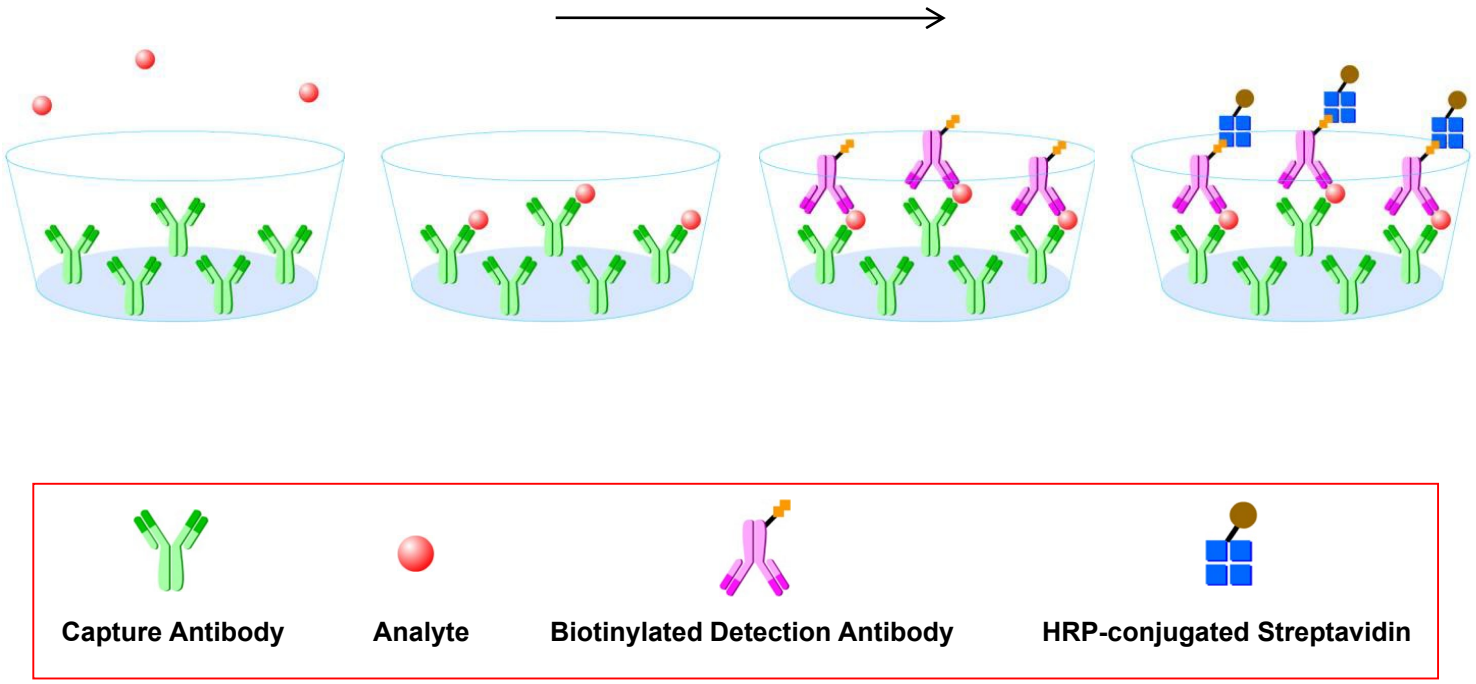
PRINCIPLE OF THE TEST

The Novus Biologicals IGF-I R/IGF1R Antibody Pair [Biotin] is a solid phase sandwich ELISA (Enzyme-Linked Immunosorbent Assay). It utilizes a monoclonal antibody specific for IGF-I R/IGF1R coated on a 96-well plate. Standards and samples are added to the wells, and any IGF-I R/IGF1R present binds to the immobilized antibody. The wells are washed and a biotinylated rabbit anti-IGF-I R/IGF1R polyclonal antibody is then added, producing an antibody-antigen-antibody "sandwich". To produces color in proportion to the amount of IGF-I R/IGF1R present in the sample strepavidin-HRP and TMB substrate solution are loaded. The absorbances of the microwell are read at 450 nm.

INTENDED USE

- The human IGF-I R/IGF1R Antibody Pair [Biotin] is for the quantitative determination of human IGF-I R/IGF1R.
- This IGF-I R/IGF1R Antibody Pair [Biotin] contains the basic components required for the development of sandwich ELISAs.

ASSAY PROCEDURE SUMMARY



This antibody pair has been configured for research use only and is not to be used in diagnostic procedures.

MATERIALS PROVIDED

Bring all reagents to room temperature before use.

Capture Antibody - 0.5 mg/mL of mouse anti-IGF-I R/IGF1R monoclonal antibody. Dilute to a working concentration of 1 µg/mL in CBS before coating.

Detection Antibody - Each vial contains 35µg biotinylated rabbit anti-IGF-I R/IGF1R polyclonal antibody. Reconstitute with 1 mL detection antibody dilution buffer. After reconstitution, store at -20℃ to -80℃ in a manual defrost freezer. Dilute to a working concentration of 0.25 µg/mL in detection antibody dilution buffer before use

Standard - Each vial contains 140ng of recombinant IGF-I R/IGF1R. Reconstitute with 1 mL detection antibody dilution buffer. After reconstitution, store at -20℃ to -80℃ in a manual defrost freezer. A seven-point standard curve using 2-fold serial dilutions in sample dilution buffer, and a high standard of 10ng/mL is recommended

Streptavidin-HRP - 50 µL of streptavidin conjugated to horseradish-peroxidase. 1:2000 Dilution in detection antibody dilution buffer before use

SOLUTIONS REQUIRED

CBS - 0.05M Na_2CO_3 , $NaHCO_3$, pH 9.6, 0.2µm filtered

TBS - 25mM Tris, adjust pH to 7.4 by HCl

Wash Buffer - 0.05% Tween20 in TBS, pH 7.2 - 7.4

Blocking Buffer - 2% BSA in Wash Buffer

Sample dilution buffer - 0.1% BSA in wash buffer, pH 7.2 - 7.4, 0.2µm filtered

Detection antibody dilution buffer - 0.5% BSA in wash buffer, pH 7.2 - 7.4, 0.2µm filtered.

Substrate Solution : To achieve best assay results, fresh substrate solution is recommended

Substrate stock solution - 10mg / ml TMB (Tetramethylbenzidine) in DMSO

Substrate dilution buffer - 0.05M Na_2HPO_4 and 0.025M citric acid ; adjust pH to 5.5

Substrate working solution - For each plate dilute 250ul substrate stock solution in 25ml substrate dilution buffer and then add 80ul 0.75% H_2O_2 , mix it well

Stop Solution - 2 N H_2SO_4

PRECAUTION

The Stop Solution suggested for use with this antibody pair is an acid solution. Wear eye, hand, face, and clothing protection when using this material.

STORAGE

Capture Antibody: Aliquot and store at -20℃ to -80℃ for up to 6 months from date of receipt. Avoid repeated freeze-thaw cycles.

Detection Antibody: Aliquot and store at -20℃ to -80℃ for up to 6 months from date of receipt. Avoid repeated freeze-thaw cycles.

Standard: Store lyophilized standard at -20℃ to -80℃ for up to 6 months from date of receipt. Aliquot and store the reconstituted standard at -20℃ to -80℃ for up to 1 month. Avoid repeated freeze-thaw cycles.

Streptavidin-HRP: Store at 4℃ and protect it from prolonged exposure to light. **DO NOT FREEZE!** It is stable for up to 6 months from date of receipt.

GENERAL ELISA PROTOCOL

Plate Preparation

1. Dilute the capture antibody to the working concentration in CBS. Immediately coat a 96-well microplate with 100μL per well of the diluted capture antibody. Seal the plate and incubate overnight at 4℃.
2. Aspirate each well and wash with at least 300μl wash buffer, repeating the process two times for a total of three washes. Complete removal of liquid at each step is essential for good performance. After the last wash, remove any remaining wash buffer by inverting the plate and blotting it against clean paper towels.
3. Block plates by adding 300 μL of blocking buffer to each well. Incubate at room temperature for a minimum of 1 hour.
4. Repeat the aspiration/wash as in step 2. The plates are now ready for sample addition.

Assay Procedure

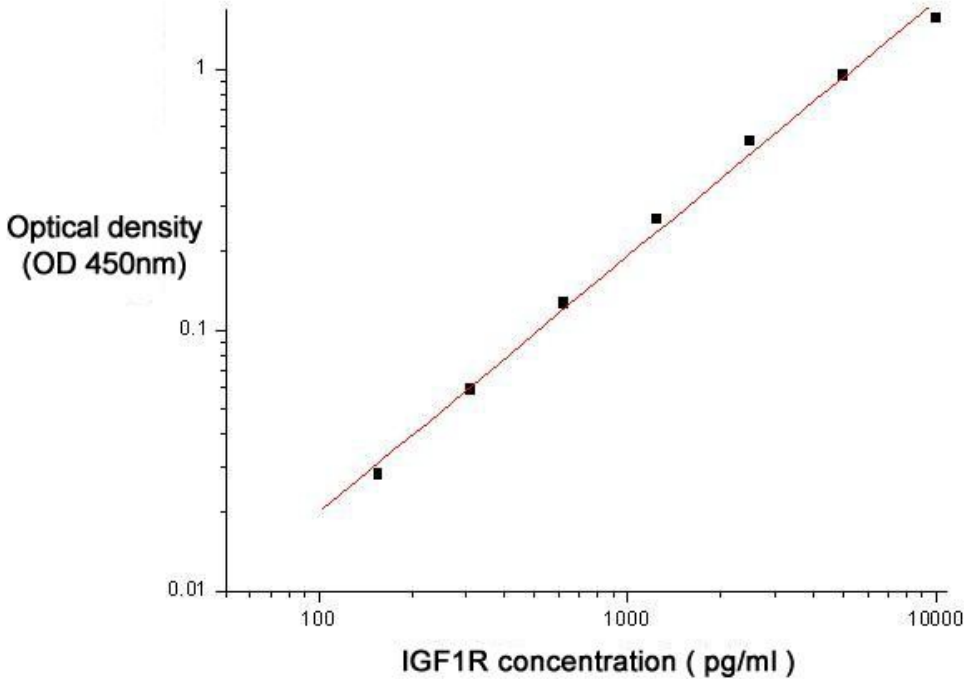
1. Add 100 μL of sample or standards in sample dilution buffer per well. Seal the plate and incubate 2 hours at room temperature.
2. Repeat the aspiration/wash as in step 2 of plate preparation.
3. Add 100 μL of the detection antibody, diluted in antibody dilution buffer, to each well. Seal the plate and incubate 1 hour at room temperature.
4. Repeat the aspiration/wash as in step 2 of plate preparation.
5. Add 100 μL of Streptavidin-HRP to each well. Incubate for 1 hour at room temperature.
6. Repeat the aspiration/wash as in step 2 of plate preparation.
7. Add 200 μL of substrate solution to each well. Incubate for 20 minutes at room temperature **(if substrate solution is not as requested, the incubation time should be optimized)**. Avoid placing the plate in direct light.
8. Add 50 μL of stop solution to each well. Gently tap the plate to ensure thorough mixing.
9. Determine the optical density of each well immediately, using a microplate reader set to 450 nm.

CALCULATION OF RESULTS

- Calculate the mean absorbance for each set of duplicate standards, controls and samples. Subtract the mean zero standard absorbance from each.
- Construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph.
- To determine the concentration of the unknowns, find the unknowns' mean absorbance value on the y-axis and draw a horizontal line to the standard curve. At the point of intersection, draw a vertical line to the x-axis and read the concentration. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.
- Alternatively, computer-based curve-fitting statistical software may also be employed to calculate the concentration of the sample.

TYPICAL DATA

This standard curve is only for demonstration purposes. A standard curve should be generated for each assay.



Concentration (pg/ml)	Zero standard subtracted OD
0	0.000
156	0.028
312.5	0.059
625	0.127
1250	0.266
2500	0.528
5000	0.943
10000	1.568

PERFORMANCE CHARACTERISTIC

SENSITIVITY

The minimum detectable dose of human IGF-I R/IGF1R was determined to be approximately **156 pg/ml**. This is defined as at least three times standard deviations above the mean optical density of 10 replicates of the zero standard.

TROUBLE SHOOTING

Problems	Possible Sources	Solutions
No signal	Incorrect or no Detection Antibody was added	Add appropriate Detection Antibody and continue
	Substrate solution was not added	Add substrate solution and continue
	Incorrect storage condition	Check if the kit is stored at recommended condition and used before expiration date
Poor Standard Curve	Standard was incompletely reconstituted or was inappropriately stored	Aliquot reconstituted standard and store at -80 ℃
	Imprecise / inaccurate pipetting	Check / calibrate pipettes
	Incubations done at inappropriate temperature, timing or agitation	Follow the general ELISA protocol
	Background wells were contaminated	Avoid cross contamination by using the sealer appropriately
Poor detection value	The concentration of antigen in samples was too low	Enriching samples to increase the concentration of antigen
	Samples were ineffective	Check if the samples are stored at cold environment. Detect samples in timely manner
High Background	Insufficient washes	Use multichannel pipettes without touching the reagents on the plate
		Increase cycles of washes and soaking time between washes
	TMB Substrate Solution was contaminated	TMB Substrate Solution should be clear and colorless prior to addition to wells
	Materials were contaminated.	Use clean plates, tubes and pipettes tips
Non-specificity	Samples were contaminated	Avoid cross contamination of samples
	The concentration of samples was too high	Try higher dilution rate of samples

ELISA Plate Template												
	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

Human IGF-I R/IGF1R Antibody Pair [Biotin]

Notes