



IGF-1R [pYpY1135/1136] Antibody Bead Kit

INFORMATION SHEET

Catalog #: LHO0501 Description: IGF-1R [pYpY1135/1136] Antibody Bead Kit Lot:* 314332

*Note: A letter at the end of the lot number signifies an additional packaging of this same lot.

Intended Use

This reagent set comprises the analyte specific components for the measurement of IGF-1R [pYpY1135/1136] in cell lysates and tissue homogenates. Buffer reagents needed to complete the reaction are sold separately under Catalog # LHB0002. These reagents are intended for use in the **Luminex 100™ or 200™ System**.

Note: This kit uses a Co-Incubation assay format. See Protocol Booklet supplied in the Intracellular Protein Buffer Reagent Kit (Cat # LHB0002) for procedure.

Note: Since Receptor Tyrosine Kinases share several conserved phosphorylation sites, when multiplexing with other RTK single-plex assays, the specific phosphorylation-site detector antibody in this assay may recognize the same phosphorylation site on other RTK proteins. Results obtained from the single-plex may vary from those obtained when multiplexing.

This antibody bead kit has been validated for multiplexing with the following antibody bead kits available from Invitrogen:

IR [pYpY1162/1163] (Cat # LHR9131)	c-Kit [pY823] (Cat # LHO0401)
EGFR [pY1068] (Cat # LHR9081)	c-Met [pY1349] (Cat # LHO0611)
Akt [pS473] (Cat # LHO0101)	p70S6K [pTpS421/424] (Cat # LHO0191)
GSK-3β [pS9] (Cat # LHO0461)	ERK1/2 [pTpY185/187] (Cat # LHO0241)*
IRS-1 [pS312] (Cat # LHO0521)	PRAS40 [pT246] (Cat # LHO0421)

* **ERK1/2 [pTpY185/187] requires Sample Treatment.** See Protocol Booklet supplied in the Intracellular Protein Buffer Reagent Kit (Cat # LHB0002) for procedure.

Before attempting to multiplex this kit with any of the other kits listed above, please contact Technical Support.

This kit is configured for research use only and is not to be used in diagnostic procedures.

Reagents Provided

1. Antibody Bead Concentrate (10x):

IGF-1R [pYpY1135/1136]

Catalog #: LM115 Description: Antibody Bead Concentrate (10x) Lot: 314333 Size: 0.25 mL-100 tests
Bead Region: 73
Form: 0.25 mL 10x bead concentrate solution in storage buffer. Contains 7.5 mM sodium azide as a preservative.
Storage: Light sensitive material. Store at 2 - 8°C, in the dark, until the expiration date indicated on the kit.

2. Detection Antibody Concentrate (10x):

IGF-1R [pYpY1135/1136]

Catalog #: DN115 Description: Detector Antibody Concentrate (10x) Lot: 314335 Size: 0.50 mL-100 tests
Form: 0.500 mL of a 10x stock of Detector Antibody Concentrate. Contains 15 mM sodium azide as preservative. Concentration of antibody is matched to this lot of beads. Do not mix lots of Coated Beads and Detector Antibody.
Storage: Store at 2 - 8°C until the expiration date indicated on the kit.

3. Standard (2 vials):

Catalog #: SM048 Description: RTK Phospho 5-Plex Standard Lot: R091413 Size: 2 vials

Form: This standard (lyophilized recombinant proteins) is designated in units/mL. The proteins in this standard have been calibrated with the respective Invitrogen ELISA kits when available. Detailed information on calibration is provided on the accompanying page.

Storage: Store at 2 - 8°C. Use within 1 hour after reconstitution. The remaining reconstituted standard should be discarded or frozen in aliquots at -80°C for further use. The standard can be frozen and thawed one time without loss of immunoreactivity.

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Concentration of Reconstituted Standards**:

IR [pYpY1162/1163] (200 units/mL) c-Kit [pY823] (200 units/mL)
IGF-1R [pYpY1135/1136] (475 units/mL) c-Met [pY1349] (200 units/mL)
EGFR [pY1068] (200 units/mL)

****Important note:** The concentrations of reconstituted standards are lot-specific. Please verify all concentration values entered in data analysis software.

Reconstitution: Reconstitute in **0.5 mL Assay Diluent**.

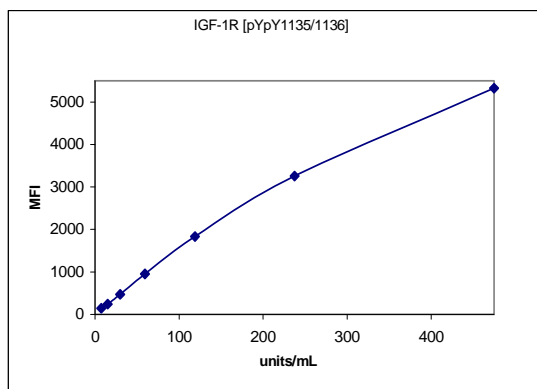
Calibration: This assay was calibrated to the Invitrogen Akt Pathway Phospho 7-Plex kit (Cat # LHO0001).

Calibration Equation: This assay was calibrated to the IGF-1R [pYpY1135/1136] Invitrogen ELISA kit (Cat # KHO0501). It is correlated by a specific multiplication factor.

Value of Standard (See Concentration of Reconstituted Standard above) x Multiplication Factor* (See Table below) = ELISA Value (Units/mL)

Performance Characteristics

Typical Standard Curve



Protein	IGF-1R [pYpY1135/1136]
Sensitivity (units/mL) ¹	1.3
Recovery (10% FNN0011) ²	86%
Recovery (10% FNN0021) ²	95%
Intra-assay variation (n=16)	5.4%
Inter-assay variation (n=32)	6.6%
(Multiplication Factor)*	0.25

Note: This is a representative standard curve.
Absolute MFI values will vary.

¹**Analytical Sensitivity:** The analytical sensitivity was determined by adding two standard deviations to the median fluorescence intensity obtained when the zero standard was assayed 32 times.

²**Recovery:** To evaluate recovery, analytes were spiked at 3 different concentrations into 10% Cell Extraction Buffer (Invitrogen Cat. # FNN0011) and 10% NP40 Cell Lysis Buffer (Invitrogen Cat. # FNN0021).

Species Reactivity: This reagent set recognizes human, mouse, and rat proteins. Other species have not been tested.

Sample Preparation: This kit has been validated with cell lysates prepared in Cell Extraction Buffer (Invitrogen Cat. # FNN0011) and NP40 Cell Lysis Buffer (Invitrogen Cat. # FNN0021). Optimization of cell stimulation and cell lysis procedures may be required for each specific application. Refer to Product Insert for detailed instructions.

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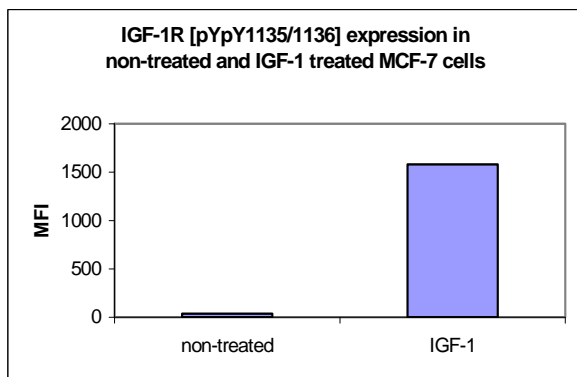
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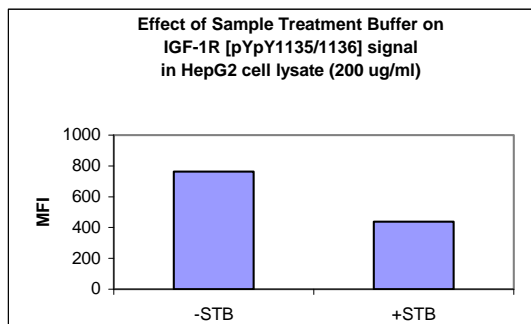
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Example Data: To evaluate the performance of this kit, MCF-7 cells were serum starved for 16 hours in the presence of 1 mM sodium vanadate and either left untreated, or treated with 100 ng/mL IGF-1 for 15 minutes. The results indicated that phosphorylation of IGF-1R at tyrosine 1135 and 1136 increased following IGF-1 treatment.



Important Note: With some of the bead immunoassay kits available from Invitrogen, cell lysates must be pre-incubated in *Sample Treatment Buffer* (Invitrogen Cat. # ST001) to optimize signal. This sample pre-incubation step has been found to adversely impact the signal obtained with other kits. The impact of the *Sample Treatment Buffer* pre-incubation step must therefore be considered when developing multiplexed assays for the detection of multiple markers with these reagents.

The data presented below demonstrate the impact of the *Sample Treatment Buffer* pre-incubation step on the observed signal. In this study, IGF1 stimulated HepG2 cells were lysed in Cell Extraction Buffer. Lysates were either treated with *Sample Treatment Buffer* (+STB) or the *Sample Treatment Buffer* incubation step was omitted (-STB).



By purchasing this Kit, which contains fluorescently labeled microsphere beads authorized by Luminex® Corporation ("Luminex®"), you, the customer, acquire the right under Luminex's patent rights to use this Kit or any portion of this Kit, including without limitation the microsphere beads contained herein, only with Luminex's laser based fluorescent analytical test instrumentation marketed under the name Luminex® 100™ or 200™. This product is covered by one or more of the following U.S. patents: 6,046,807.

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