

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

Intended Use

This reagent set comprises of components for the measurement of the phosphorylated proteins IR[pYpY1162/1163], IGF-1R[pYpY1135/1136], IRS-1[pS312], Akt[pS473], PRAS40[pT246], p70S6K[pTpS421/424], and GSK-3β[pS9] in cell lysates and tissue homogenates. These reagents are intended for use in the **Luminex® 100™ or 200™ System**.

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

Reagents Provided

Akt Pathway Phospho 7-Plex					
1.	Part #: LM114	Description: Antibody Bead Concentrate (10X)	Lot: 737535	Size: 0.25 mL-100 tests	
	Analyte:	Bead Region:	Analyte:	Bead Region:	
	IR [pYpY1162/1163]	72	PRAS40 [pT246]	74	
	IGF-1R [pYpY1135/1136]	73	p70S6K [pTpS421/424]	58	
	IRS-1 [pS312]	75	GSK-3β [pS9]	59	
	Akt [pS473]	14			

Form: 0.25 mL 10X bead concentrate solution in storage buffer. Contains 0.05% sodium azide as a preservative.

Storage: **Light sensitive material.** Store at 2 to 8°C, in the dark, until the expiration date indicated on the kit.

Recommended Dilution: Dilute 2.5 µL Antibody Bead Concentrate in 25 µL Working Wash Solution per assay well. Use 25 µL of diluted bead solution per well. Refer to the User Manual included in the kit for further information.

Akt Pathway Phospho 7-Plex				
2.	Part #: <u>DN114</u>	Description: <u>Detection Ab Concentrate (10X)</u>	Lot: <u>737534</u>	Size: <u>0.5 mL-100 tests</u>
Form:	0.500 mL of a 10X stock of Detector Antibody Concentrate. Contains 0.1% 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 to 8°C until the expiration date indicated on the kit.			

Recommended Dilution: Dilute 5 µL Detector Antibody Concentrate in 50 µL *Detector Antibody Diluent* per assay well. Use 50 µL of diluted detector antibody per well. Refer to the User Manual included in the kit for further information.

3.	Part #: <u>SM040</u>	Description: <u>Akt Pathway Phospho 7-Plex Standard</u>	Lot: <u>774545</u>	Size: <u>2 Vials</u>	
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. Contains 0.1% sodium azide as a preservative.				
Storage:	Store at 2 to 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.				

Concentration of Reconstituted Standards**:

IR [pYpY1162/1163] (830 units/mL)	PRAS40 [pT246] (700 units/mL)
IGF-1R [pYpY1135/1136] (660 units/mL)	p70S6K [pTpS421/424] (320 units/mL)
IRS-1 [pS312] (430 units/mL)	GSK-3β [pS9] (160 units/mL)
Akt [pS473] (140 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*.

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Akt Pathway Phospho 7-Plex Panel

Technical Data Sheet

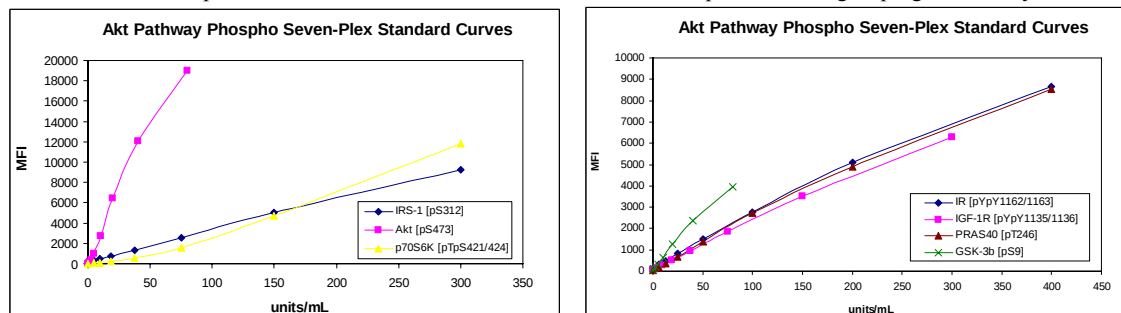
Recommended Starting Concentration for Standard Curve: Upon reconstitution, the starting concentration of standards is the value cited above. Make 2-fold serial dilutions in Assay Diluent. Refer to the User Manual included in the kit for further information.

Calibration: The following markers were calibrated to Invitrogen ELISA kits (catalog numbers): IR [pYpY1162/1163] (KHR9131), Akt [pS473] (KHO0111), PRAS40 [pT246] (KHO0421), and GSK-3 β [pS9] (KHO0461). The p70S6K [pTpS421/424] was calibrated to Invitrogen Luminex kit, catalog number LHO0191. One unit of IRS-1 [pS312] is equivalent to 150 pg recombinant IRS-1 that was phosphorylated by JNK2 α 1 (Invitrogen Cat # PHO3035). One unit of IGF-1R [pYpY1135/1136] is equivalent to 17 pg autophosphorylated recombinant IGF-1R. Subsequent lots of standard will be normalized to this lot of material to allow consistency of IRS-1 [pS312] and IGF-1R [pYpY1135/1136] quantitation.

Performance Characteristics

Typical Standard Curves

The standard curves presented here were collected as a 7-Plex, but are depicted in two groupings for clarity.



Protein	IR [pYpY1162/1163]	IGF-1R [pYpY1135/1136]	IRS-1 [pS312]	Akt [pS473]	PRAS40 [pT246]	p70S6K [pTpS421/424]	GSK-3 β [pS9]
Sensitivity (units/mL) ¹	<3	<2.4	<4	<0.25	<2	<7	<1
Recovery (10% FNN0011) ²	88%	103%	92%	100%	89%	95%	99%
Recovery (10% FNN0021) ²	102%	104%	99%	109%	98%	103%	101%
Intra-assay variation (n=14)	5.6%	7.3%	5.7%	6.0%	6.3%	6.1%	4.5%
Inter-assay variation (n=32)	6.1%	7.9%	6.5%	7.4%	7.4%	6.5%	5.9%

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: Each analyte specific component of this reagent set recognizes human, mouse, and rat proteins. Other species have not been tested.

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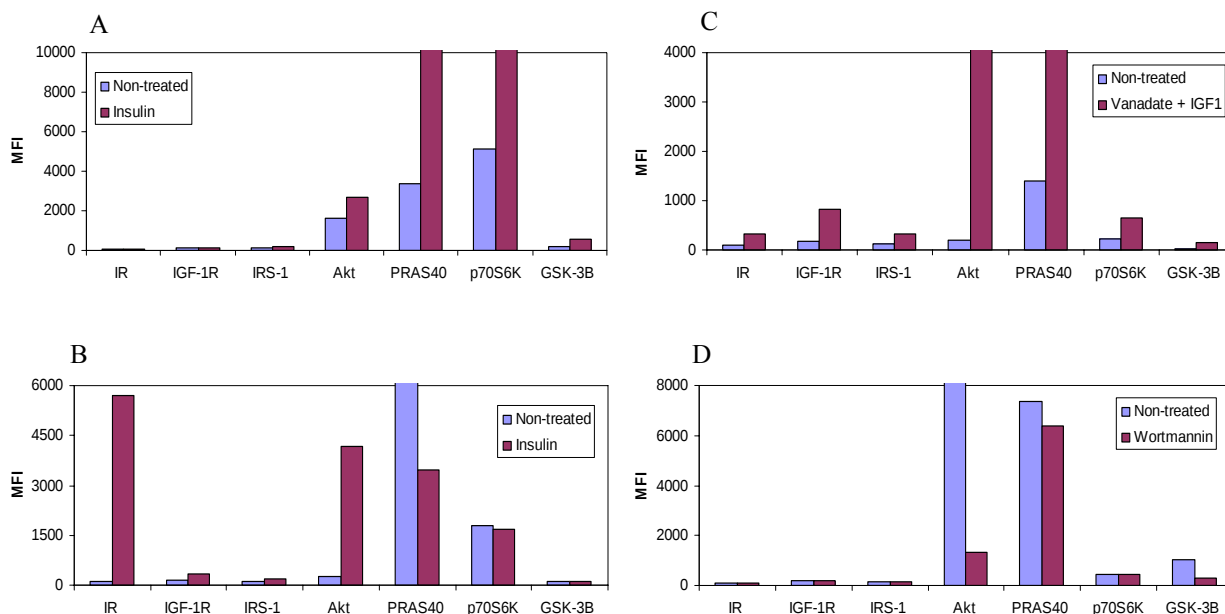
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Akt Pathway Phospho 7-Plex Panel

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Specificity: The analytes of the multiplex were assayed individually with the Akt Pathway Phospho 7-Plex and were all found to have no cross-reactivity.

To further evaluate the performance of this kit, MCF7 cells (Figure A) and IR-transfected CHO-T cells (Figure B) were treated with insulin. Cells were serum starved for 15 hours and either left untreated, or treated with 100 nM of insulin for 15 minutes. In figure C, HepG2 cells were stimulated by IGF1 in the presence of sodium vanadate. In this study, HepG2 cells were serum starved for 15 hours and either left untreated, or treated with 100 ng/mL of IGF1 for 15 minutes. In figure D, Jurkat cells were treated with Wortmannin, a PI3 kinase inhibitor. The cells were either left untreated, or treated with 200 nM of Wortmannin for 3 hours.



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 User Manual for detailed instructions.

Explanation of symbols

Symbol	Description	Symbol	Description
	Catalogue Number		Batch code
	Research Use Only		In vitro diagnostic medical device
	Use by		Temperature limitation
	Manufacturer		European Community authorised representative
	Without, does not contain		With, contains
	Protect from light		Consult accompanying documents
	Directs the user to consult instructions for use (IFU), accompanying the product.		

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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