



# AKT Pathway Phospho 7-Plex Panel

## Technical Data Sheet

Catalog #: LHO0001

Lot #:\* 1001253

\*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-3 $\beta$ [pS9] in cell lysates and tissue homogenates. These reagents are intended for use in the **Luminex<sup>®</sup> 100<sup>™</sup>/200<sup>™</sup>** and the **FLEXMAP 3D<sup>®</sup> 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. | <b>Part #:</b> LM114 | <b>Description:</b> Antibody Bead Concentrate (10X)  | <b>Lot:</b> 1018739 | <b>Size:</b> 0.25 mL-100 tests |
|    | <b>Form:</b>         | 0.25 mL 10X bead concentrate solution in storage buffer. Contains 0.05% sodium azide as a preservative.  |                     |                                |
|    | <b>Storage:</b>      | <b>Light sensitive material.</b> Store at 2 to 8°C, in the dark, until the expiration date indicated on the kit.   |                     |                                |
|    |                      | Akt Pathway Phospho 7-Plex   |                     |                                |
| 2. | <b>Part #:</b> DN114 | <b>Description:</b> Detection Ab Concentrate (10X)   | <b>Lot:</b> 1018737 | <b>Size:</b> 0.5 mL-100 tests  |
|    | <b>Form:</b>         | 0.5 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.                  |                     |                                |
|    | <b>Storage:</b>      | Store at 2 to 8°C until the expiration date indicated on the kit.  |                     |                                |
| 3. | <b>Part #:</b> SM040 | <b>Description:</b> Akt Pathway Phospho 7-Plex Standard  | <b>Lot:</b> 1007684 | <b>Size:</b> 2 Vials           |
|    | <b>Form:</b>         | Lyophilized. The proteins in this standard have been calibrated against the masses of highly purified recombinant proteins, with the respective Invitrogen ELISA kits when available. Contains 0.1% sodium azide as a preservative.    |                     |                                |
|    | <b>Storage:</b>      | Store at 2 to 8°C. Use within 1 hour after reconstitution. The remaining 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 Standard\*\*:

IR [pYpY1162/1163] (1609 units/mL)	PRAS40 [pT246] (997 units/mL)
IGF-1R [pYpY1135/1136] (1274 units/mL)	p70S6K [pTpS421/424] (518 units/mL)
IRS-1 [pS312] (503 units/mL)	GSK-3 $\beta$ [pS9] (68 units/mL)
Akt [pS473] (105 units/mL)	

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

**Reconstitution:** Reconstitute in **1.0 mL** Assay Diluent.

**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<sup>™</sup> ELISA kits (catalog numbers): IR [pYpY1162/1163] (KHR9131), Akt [pS473] (KHO0111), PRAS40 [pT246] (KHO0421), and GSK-3 $\beta$  [pS9] (KHO0461). The p70S6K [pTpS421/424] was calibrated to Invitrogen<sup>™</sup> Luminex singleplex kit, catalog number LHO0191. One unit of IRS-1 [pS312] is equivalent to 150 pg recombinant IRS-1 that was phosphorylated by JNK2 $\alpha$ 1 (Invitrogen<sup>™</sup> 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.

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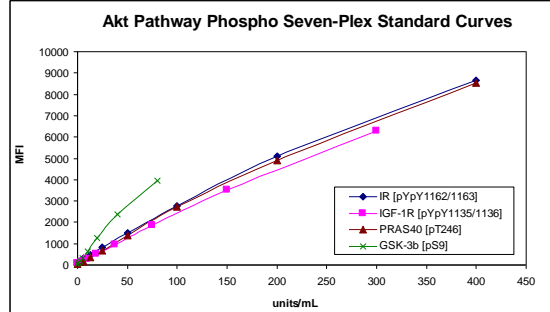
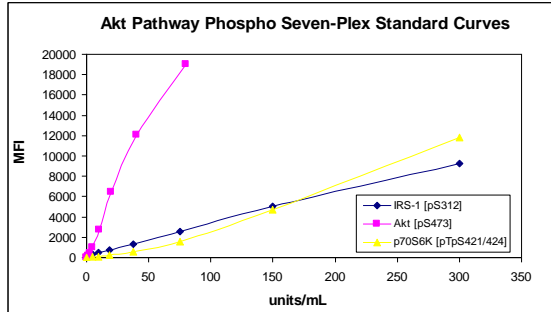
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### Performance Characteristics

#### Typical Standard Curves

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



Protein	Akt [pS473]	p70S6K [pTpS421/424]	GSK-3β [pS9]	IR [pYpY1162/1163]	IGF-1R [pYpY1135/1136]	PRAS40 [pT246]	IRS-1 [pS312]
Bead Region	14	58	59	72	73	74	75
Sensitivity (units/mL) <sup>1</sup>	< 0.5	< 0.5	< 0.5	< 1	< 5	< 0.5	< 0.5
Recovery (10% FNN0011) <sup>2</sup>	100%	95%	99%	88%	103%	89%	92%
Recovery (10% FNN0021) <sup>2</sup>	109%	103%	101%	102%	104%	98%	99%
Intra-assay variation (n=14)	6.0%	6.1%	4.5%	5.6%	7.3%	6.3%	5.7%
Inter-assay variation (n=32)	7.4%	6.5%	5.9%	6.1%	7.9%	7.4%	6.5%

<sup>1</sup>**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.

<sup>2</sup>**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.

**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.

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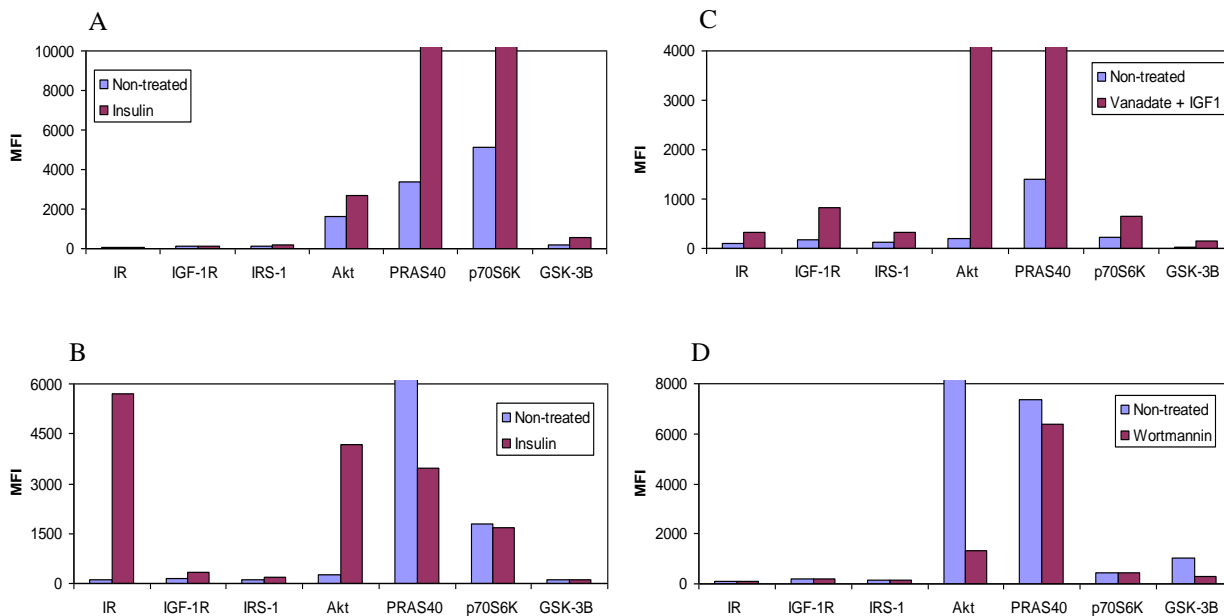
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To further evaluate the performance of this kit, MCF7 cells (Figure A) and IR-transfected CHO-T cells (Figure B) were treated with insulin. Cells were serum starved for 15 hours and either left untreated, or treated with 100 nM of insulin for 15 minutes. 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 to the user manual for detailed instructions.

### Explanation of symbols

Symbol	Description	Symbol	Description
	Catalogue Number		Batch code
	Research Use Only		<i>In vitro</i> 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.		

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