



Akt Pathway Total 7-Plex Panel

Technical Data Sheet

Catalog #: LHO0002 Lot #:* 1223602

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

Product Use

This kit is comprised of components for the measurement of IR, IGF-1R, IRS-1, Akt, PRAS40, p70S6K, and GSK-3 β in cell lysates and tissue homogenates, independent of their phosphorylation states. These reagents are intended for use in the **Luminex[®] 100[™]/200[™]** and the **FLEXMAP 3D[®] System**. This kit is configured for research use only and is not to be used in diagnostic procedures.

Reagents Provided

1. Part #: LM128 Description: AKT Pathway Total 7-Plex Antibody Bead Concentrate (10X) Lot: 1223601 Size: 0.25 mL-100 tests

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.

2. Part #: DN128 Description: AKT Pathway Total 7-Plex Detection Ab Concentrate (10X) Lot: 999387 Size: 0.5 mL-100 tests

Form: 0.50 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.

3. Part #: SM043 Description: AKT Pathway (Total) 7-Plex Standard Lot: 1007685 Size: 2 Vials

Form: 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.

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 Standard**:

Akt (33.63 ng/mL)	p70S6K (43.29 ng/mL)	GSK-3 β (25.94 ng/mL)	IR (274.35 ng/mL)
IGF-1R (14.77 ng/mL)	PRAS40 (34.43 ng/mL)	IRS-1 (15.78 ng/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 standard 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 (KHR9111), Akt (KHO0101), PRAS40 (KHO0411), and GSK-3 β (KHO0451). The IGF-1R, IRS-1 and p70S6K were calibrated to the mass of highly purified recombinant proteins. Subsequent lots of standard will be normalized to this lot of material to allow consistency of quantitation.

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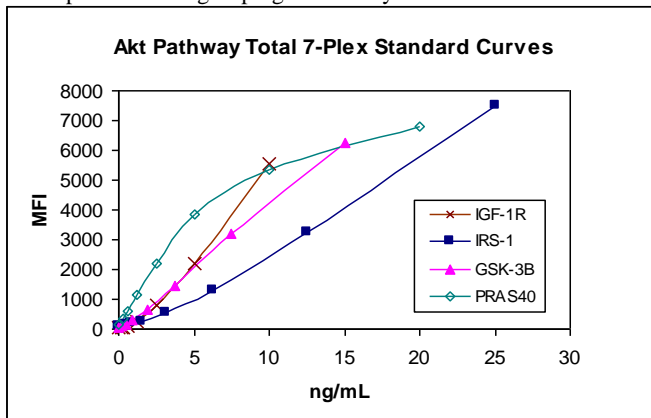
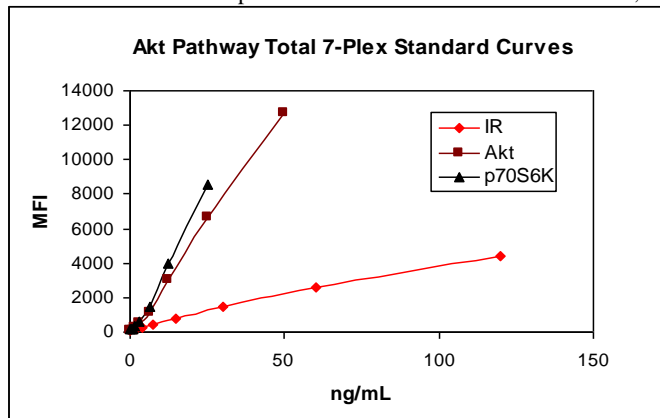
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Performance Characteristics

Typical Standard Curves

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



Analyte	Akt	p70S6K	GSK-3β	IR	IGF-1R	PRAS40	IRS-1
Bead Region	14	58	59	72	73	74	75
Sensitivity (ng/mL) ¹	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5
Recovery (10% FNN0011) ²	82%	101%	81%	80%	85%	80%	91%
Recovery (10% FNN0021) ²	94%	102%	90%	92%	98%	92%	96%
Intra-assay variation (n=14)	4.0%	6.7%	5.0%	3.5%	5.3%	4.5%	4.8%
Inter-assay variation (n=32)	3.8%	7.7%	4.6%	3.1%	4.2%	4.2%	3.8%

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.

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

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.

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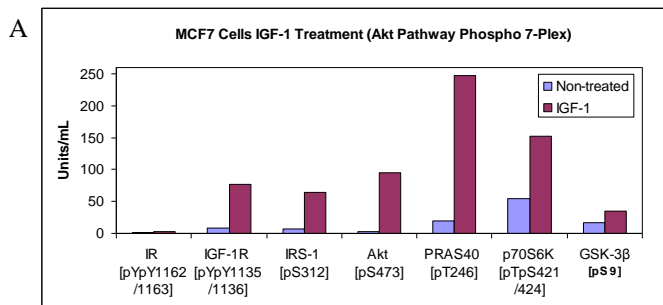
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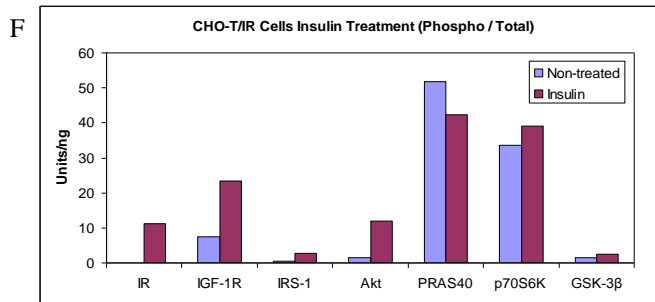
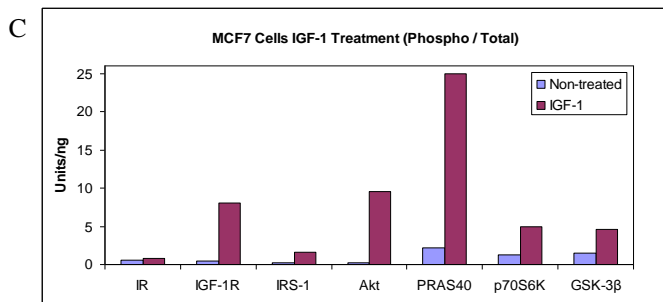
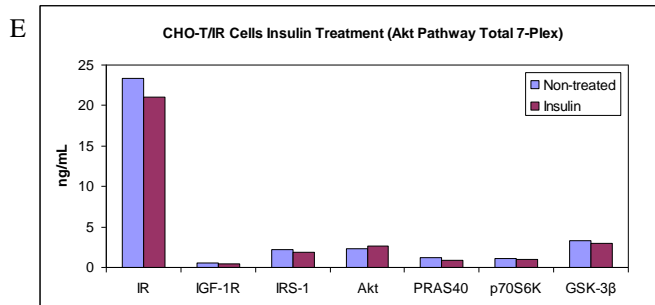
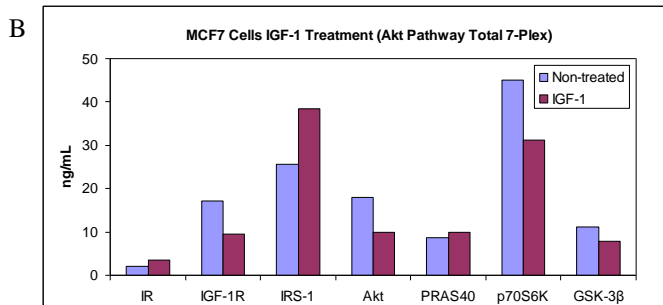
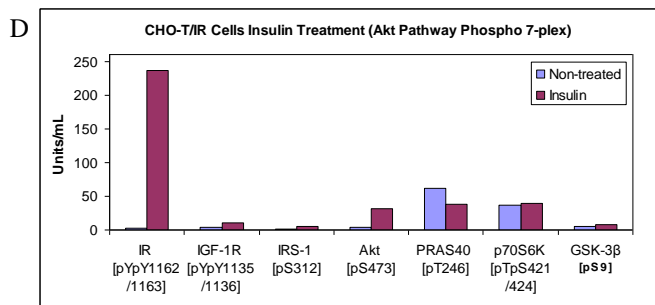
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To further evaluate the performance of this kit, MCF7 cells were treated with IGF-1 (Panel I) and IR-transfected CHO-T cells were insulin treated (Panel II). Cells were serum starved for 15 hours in the presence of sodium vanadate and either left untreated, or treated with 100 ng/mL of IGF-1 for 1 hour (Panel I) or with 100 nM of insulin for 15 minutes (Panel II). The levels of phospho (Figures A & D) (Invitrogen Cat. # LHO0001) and total (Figures B & E) proteins of the 7 analytes were determined. The data presented in Figures C & F show the results of normalizing the levels of each phospho protein to its corresponding total protein.

Panel I
MCF7 Cells IGF-1 Treatment



Panel II
CHO-T/IR Cells Insulin Treatment



Explanation of symbols

Symbol	Description	Symbol	Description	Symbol	Description
	Manufacturer		Catalog number		Batch code
	Use by		Temperature limitation		
	Consult instructions for use		Caution, consult accompanying documents		

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