

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

#### Intended Use

This kit is comprised of components for the measurement of PRAS40 [pT246] 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™/200™ and the FlexMAP 3D® System**.

**Note: This kit uses a Co-Incubation assay format.** Refer to the User Manual included in the Intracellular Protein Buffer Reagent Kit (Cat # LHB0002) for procedure.

This Singleplex Bead Kit has been validated for multiplexing with the following Singleplex Bead Kits available from Invitrogen:

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)

†ERK1/2 [pTpY185/187] requires **Sample Treatment**. Refer to the User Manual included in the Intracellular Protein Buffer Reagent Kit (Cat # LHB0002) for procedure.

#### Reagents Provided

1. **Part #:** LM118      **Description:** PRAS40 [pT246] Antibody Bead Concentrate (10X)      **Lot:** 889447      **Size:** 0.25 mL-100 tests

**Bead Region:** 74

**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 #:** DN118      **Description:** PRAS40 [pT246] Detection Ab Concentrate (10X)      **Lot:** 889446      **Size:** 0.5 mL-100 tests

**Form:** 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.

**Storage:** Store at 2 to 8°C until the expiration date indicated on the kit.

3. **Part #:** SM040      **Description:** Akt Pathway Phospho 7-Plex Standard      **Lot:** 349446      **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 the calibration is provided below. 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\*\*:

IR [pYpY1162/1163] (415 units/mL)	PRAS40 [pT246] (350 units/mL)
IGF-1R [pYpY1135/1136] (330 units/mL)	p70S6K [pTpS421/424] (160 units/mL)
IRS-1 [pS312] (215 units/mL)	GSK-3β [pS9] (80 units/mL)
Akt [pS473] (70 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 standard is the value cited above. Make serial 1:2 dilutions in Assay Diluent. Refer to the User Manual included in the buffer kit for further information.

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Invitrogen Corporation • 7335 Executive Way, Frederick, MD 21704 • Tel: 800.955.6288 • E-mail: [techsupport@invitrogen.com](mailto:techsupport@invitrogen.com)

LHO0421

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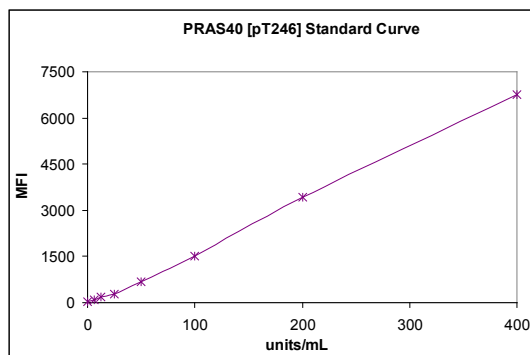
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# PRAS40 [pT246] Singleplex Bead Kit

## Technical Data Sheet

### Performance Characteristics

#### Typical Standard Curve



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

Protein	PRAS40 [pT246]
Sensitivity (units/mL) <sup>1</sup>	4.4
Recovery (10% FNN0011) <sup>2</sup>	89%
Recovery (10% FNN0021) <sup>2</sup>	98%
Intra-assay variation (n=14)	6.3%
Inter-assay variation (n=32)	7.4%

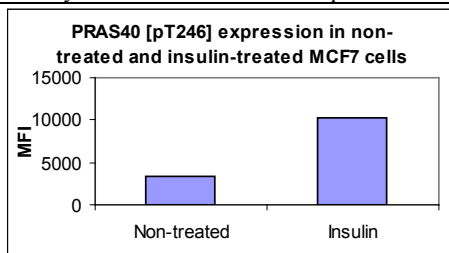
<sup>1</sup>**Analytical Sensitivity:** The analytical sensitivity was determined by adding two standard deviations to the median fluorescence intensity obtained when the zero standard was assayed 32 times.

<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).

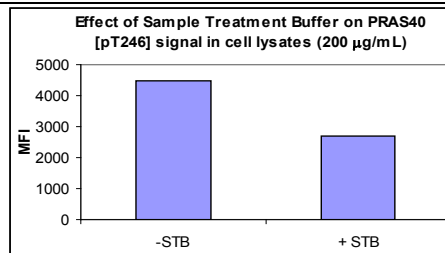
**Species Reactivity:** Each analyte specific component of this reagent set recognizes human, mouse, and rat proteins. Other species have not been tested.

**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.

**Important Note:** With some of the Singleplex bead 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.



**Example Data:** To evaluate the performance of this kit, MCF7 cells were serum starved for 15 hours and either left untreated, or treated with 100 nM insulin for 15 minutes. The results indicated that phosphorylation of PRAS40 at threonine 246 increased following insulin-treatment.



**The data presented above demonstrate the impact of the *Sample Treatment Buffer* pre-incubation step on the observed signal.** In this study, PMA-stimulated Jurkat 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).

#### Explanation of symbols

Symbol	Description	Symbol	Description
REF	Catalogue Number	LOT	Batch code
RUO	Research Use Only	IVD	<i>In vitro</i> diagnostic medical device
Hourglass	Use by	Thermometer	Temperature limitation
Factory	Manufacturer	EC REP	European Community authorised representative
[-]	Without, does not contain	[+]	With, contains
Light Protection	Protect from light	Warning	Consult accompanying documents
Book	Directs the user to consult instructions for use (IFU), accompanying the product.		

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