

*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 total p53 in cell lysates/tissue homogenates, serum and plasma. Buffer reagents needed to complete the reaction are sold separately under Catalog #LHB0002. This singleplex bead kit may be multiplexed with other phospho-specific and total protein Singleplex bead kits available from Invitrogen, but cannot be multiplexed with the p53 [pS15] Singleplex Bead Kit (Catalog #LHO0141). These reagents are intended for use in the **Luminex® 100™ or 200™ System only. This kit is configured for research use only and is not to be used in diagnostic procedures.**

Note: This kit uses the General Protocol Procedure. Refer to User Manual supplied in the Intracellular Protein Buffer Reagent Kit (Cat # LHB0002) for procedure.

Reagents Provided

1. Part #: LM091 Description: p53 (Total) Antibody Bead Concentrate (10X) Lot: 574418 Size: 0.25 mL-100 tests

Bead Region: 39

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

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

2. Part #: DN091 Description: p53 (Total) Detection Antibody Concentrate (10X) Lot: 574420 Size: 1 mL-100 tests

Form: 1 mL of a 10x stock of Detector Antibody Concentrate in Detector Antibody Diluent. 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 - 8°C until the expiration date indicated on the kit.

3. Part #: SM091 Description: Hu p53 (Total) Standard Lot: 273753 Size: 2 Vials

Form: This p53 standard (lyophilized recombinant protein) is designated in ng/mL. Contains 0.1% sodium azide as preservative. The protein in this standard has been calibrated with the respective Invitrogen ELISA kit. Detailed information on calibration is provided on the accompanying page.

Storage: Store at 2 to 8°C. Use within 1 hour after reconstitution. Discard immediately after use.

Concentration of Reconstituted Standard**:

p53 Total (8.05 ng/mL)

****Important note: The concentration of reconstituted standard is 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*. Use 100 µL per assay.

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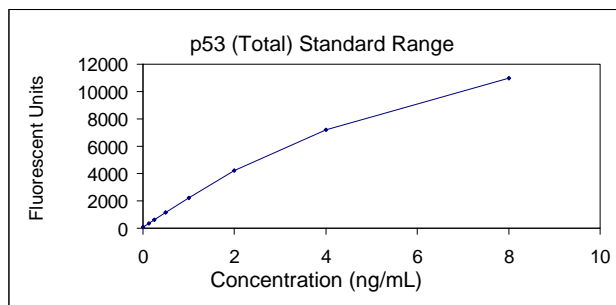
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p53 (Total) Singleplex Bead Kit

Technical Data Sheet

Performance Characteristics

Analytical Sensitivity: The analytical sensitivity of the total p53 assay is <0.05 ng/mL. This was determined by adding two standard deviations to the mean median fluorescence units obtained when the zero standard was assayed 30 times. This sensitivity corresponds to the amount of p53 extractable from approximately 1×10^4 CEM cells using NP40 Cell Lysis Buffer (formulation presented on accompanying page). The assay was found to be at least twice as sensitive as Western blotting.



Specificity: This kit is specific for p53, independent of its phosphorylation state and does not display any cross-reactivity with Akt, Bcl-2, Rb, JNK1/2, IκBα, p38 MAPK, MEK1, or STAT1.

Precision:

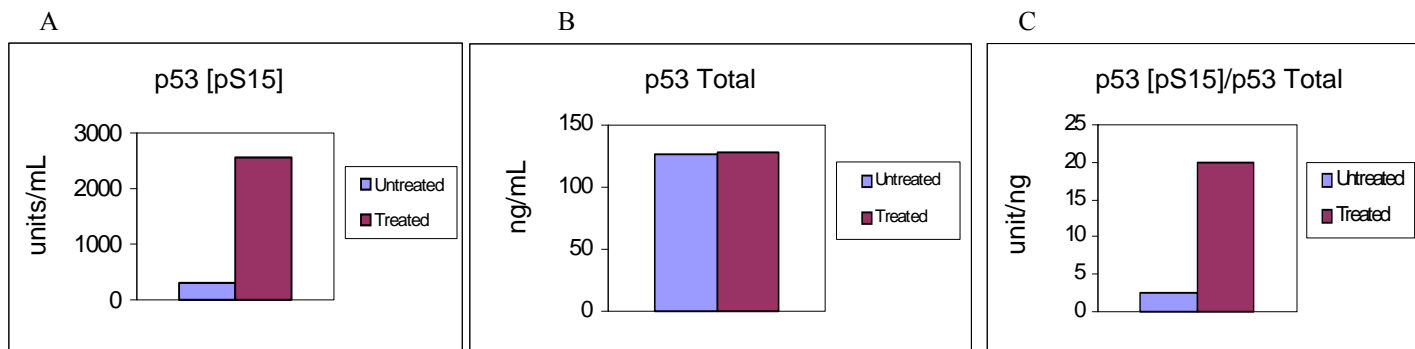
	Intra-assay (n=16)	Inter-assay (n=32)
Mean (pg/mL)	521	555
SD	16.5	38.2
%CV	3.16	6.88

Linearity of Dilution: NP40 Cell Lysis Buffer was spiked with p53 and serially diluted in *Assay Buffer* over the range of the assay. Linear regression analysis of sample values versus the expected concentration yielded a correlation coefficient of 0.99.

Recovery: To evaluate recovery, p53 was spiked at 3 different concentrations into 10% NP40 Cell Lysis Buffer. The percent recovery was calculated as an average of 117%. p53 was spiked at 3 different concentrations into human citrate plasma for an average percent recovery of 109%. p53 was also spiked into human serum at 3 different concentrations for an average recovery of 119%.

Correlation to ELISA: This assay was calibrated to the mass of highly purified recombinant p53 protein expressed in *E. coli* as well as to the Invitrogen Total p53 ELISA kit (Catalog# KHO0151). The correlation coefficient was 0.91.

To further evaluate the performance of this kit, a study using actinomycin D was undertaken. In this study, CEM cells grown in RPMI medium containing 10% FBS were either left untreated, or treated with 10 μM actinomycin D for 20 minutes at 37°C, and the levels of p53 [pS15] (Figure A) and total p53 (Figure B) were determined. This study indicated phosphorylation of p53 increased with actinomycin D treatment, while the level of total p53 remained approximately constant. The data presented in Figure C show the results of normalizing the level of p53 [pS15] to total p53.



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p53 (Total) Singleplex Bead Kit

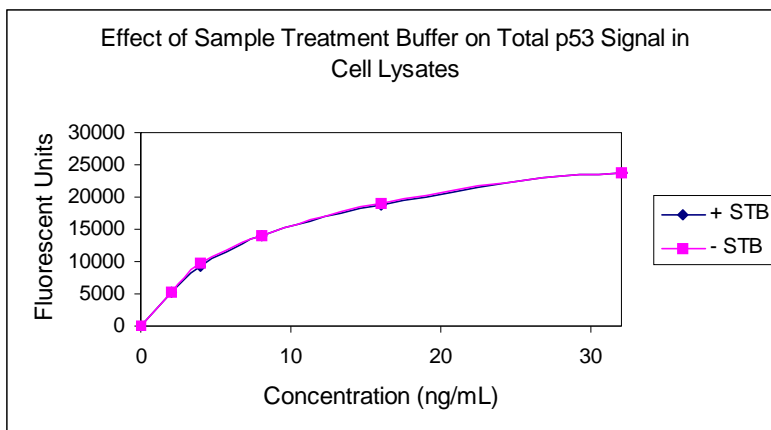
Technical Data Sheet

Sample Preparation:

This kit has been validated with cell lysates prepared in NP40 Cell Lysis Buffer (50 mM Tris, pH 7.4, 250 mM NaCl, 5 mM EDTA, 50 mM NaF, 1 mM Na₂VO₄, 1% Nonidet P40 [Roche Applied Science, Cat. # 1754599], 1 mM PMSF [stock is 0.1 M in DMSO], and protease inhibitor cocktail [Sigma Cat. # P-2714]) and diluted at least two-fold in *Assay Diluent*. To produce a lysate, incubate cells with cell lysis buffer (1-2 x 10⁸ cells/mL is recommended) on ice for 30 minutes, vortexing at 10 minute intervals, then clarify the lysate by centrifugation at 13,000 rpm for 10 minutes. Cell lysates may be stored at -80°C for up to three months with one freeze/thaw cycle. Optimization of cell stimulation and cell lysis procedures may be required for each specific application.

Important Note: With some of the Singleplex immunoassay kits available from Invitrogen, cell lysates must be pre-incubated in *Sample Treatment Buffer* 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 minimal impact of the *Sample Treatment Buffer* pre-incubation step on the observed signal. In this study, CEM cells were lysed in NP40 Cell Lysis Buffer at a concentration of 2 x 10⁸ cells/mL cell lysis buffer. Lysates were either treated with *Sample Treatment Buffer* (+STB: lysates were diluted 1:2 in *Sample Treatment Buffer*, incubated on ice for 20 minutes, diluted 1:10 in *Assay Diluent*, and then serially diluted for measurement with the kit), or the *Sample Treatment Buffer* incubation step was omitted (-STB: lysates were diluted 1:2 in NP40 Cell Lysis Buffer, then diluted 1:10 in *Assay Diluent*, and then serially diluted for measurement with the kit).



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.		

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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