



Total p53 Antibody Bead Kit

INFORMATION SHEET

Catalog #: LHO0151 **Description:** Total p53 **Lot:** Q031014 **Expiration:** 3/2005

Intended Use

This reagent set comprises the analyte specific 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 antibody bead kit may be multiplexed with other phospho-specific and total protein antibody bead kits available from BioSource, but cannot be multiplexed with the p53 [pS15] Antibody Bead Kit (Catalog #LHO0141). These reagents are intended for use in the Luminex™ 100 System only. **This kit is configured for research use only and is not to be used in diagnostic procedures.**

Reagents Provided

1. Antibody Bead Concentrate (10x):

Catalog #: LM091 **Description:** p53 Beads **Lot:** Q031017 **Size:** 0.25 mL-100 tests

Bead Region: 39

Form: 0.25 mL 10x bead concentrate solution in storage buffer. Contains 15 mM sodium azide as preservative.

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

Recommended Dilution: Use 25 µL of diluted bead solution per assay. Mix 1 part beads with 9 parts Working Wash Solution. If establishing a Multiplex Assay, mix equal volumes of different beads and adjust volume of Working Wash Solution down to remain at 25 µL/assay. See the Product Insert included in the Buffer Reagent Kit for further information.

2. Detector Antibody Concentrate (10x):

Catalog #: DN091 **Description:** Total p53 Detector **Lot:** Q031019 **Size:** 1 mL-100 tests

Form: 1 mL of a 10x stock of Detector Antibody Concentrate in Detector Antibody Diluent. Contains 15 mM 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.

Recommended Dilution: Mix 1 part Detector Antibody Concentrate with 9 parts Detector Antibody Diluent. Use 100 µL per assay. If establishing a Multiplex Assay, mix equal volumes of different Detector Antibody Concentrates and adjust the volume of Detector Antibody Diluent down to remain at 100 µL/assay. See the Product Insert included in the Buffer Reagent Kit for further information.

3. Standard (2 vials):

Catalog #: SM091 **Description:** Total p53 Standard **Lot:** Q031021 **Size:** Single use

Form: This p53 standard (lyophilized recombinant protein) is designated in ng/mL. The protein in this standard has been calibrated with the respective BioSource ELISA kit. Detailed information on calibration is provided on the accompanying page.

Storage: Store at 2 - 8°C. Use within 2 hours after reconstitution. Discard immediately after use.

Concentration of Reconstituted Standard:** p53 Total 8 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 0.7 mL Assay Diluent.

Recommended Starting Concentration for Standard Curve: Upon reconstitution, the starting concentration of standards is the value cited above. Make serial 1:2 dilutions in Assay Diluent. Use 100 µL per assay. If establishing a Multiplex Assay, this same standard can be used to measure the other related proteins cited above in a Multiplex Assay format. See the Product Insert included in the Buffer Reagent Kit for further information.

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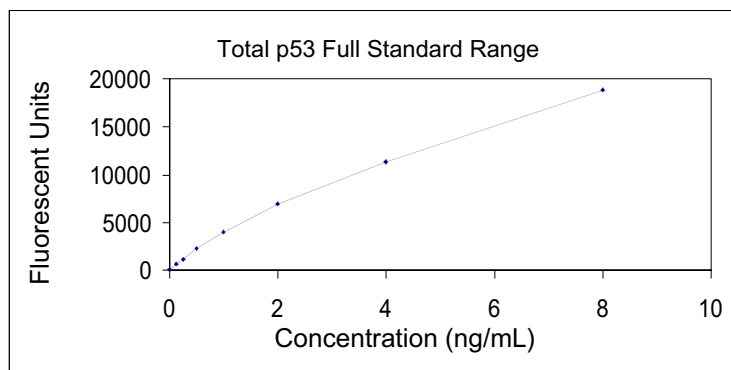
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Performance Characteristics

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 x 10⁴ 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.



Representative Standard Curve

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:

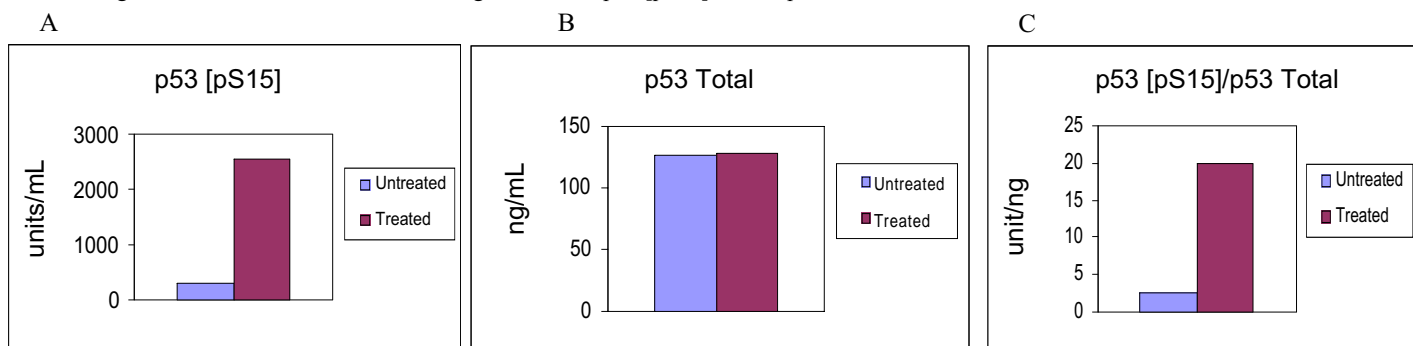
	Intra-assay (n=16)	Inter-assay (n=32)
Mean (ng/mL)	521	555
SD	16.5	38.2
%CV	3.16	6.88

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 BioSource 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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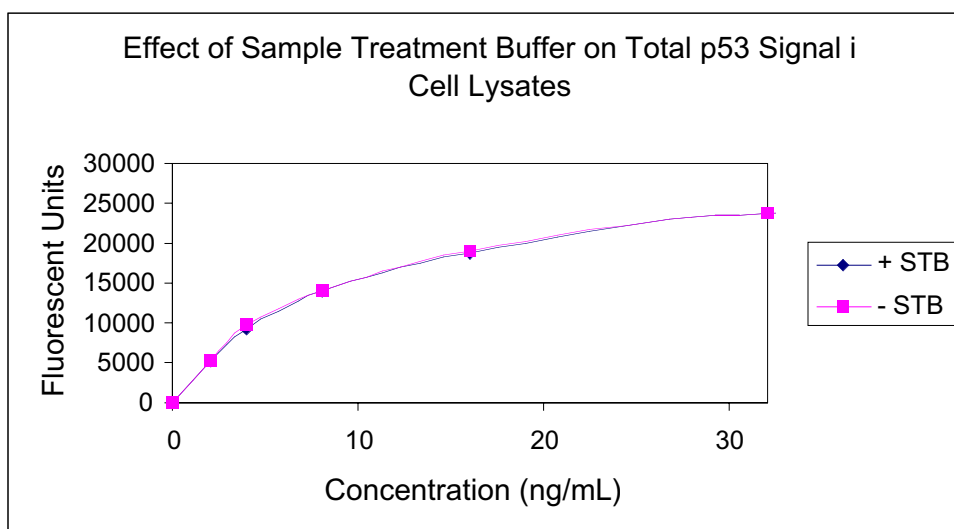
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Sample Preparation:

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 bead immunoassay kits available from BioSource, 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).



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. This product is covered by one or more of the following U.S. patents: 6,046,807.

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