Total Human Rb Antibody Bead Kit
INFORMATION SHEET

Catalog #: LHO0011 **Description:** Total Rb **Lot:*** S062010

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

Intended Use

This reagent set comprises the analyte specific components for the measurement of total human Rb in cell lysates/tissue homogenates. 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 Invitrogen, but cannot be multiplexed with the Rb [pT821] Antibody Bead Kit (Catalog #LHO0021) or the Rb [pSpT249/252] Antibody Bead Kit (Catalog #LHO0171). 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 Assay Procedure. See Protocol Booklet supplied in the Intracellular Protein Buffer Reagent Kit (Cat # LHB0002) for procedure.

Reagents Provided

1. Antibody Bead Concentrate (10x):

Catalog #: LM021 **Description:** Rb Beads **Lot:** S071206 **Size:** 0.25 mL-100 tests

Bead Region: 06
Form: 0.25 mL 10x bead concentrate solution in storage buffer. Contains 7.5 mM sodium azide as preservative.
Storage: Store at 2 to 8°C until the expiration date indicated on the kit.

2. Detector Antibody Concentrate (10x):

Catalog #: DN021 **Description:** Total Rb Detector **Lot:** S071305 **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 to 8°C until the expiration date indicated on the kit.

3. Standard (2 vials):

Catalog #: SM021 **Description:** Total Rb Standard **Lot:** S071701 **Size:** Single use

Form: This Rb standard (lyophilized Jurkat cell lysate) is designated in ng/mL. 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:** Rb Total 20 ng/mL

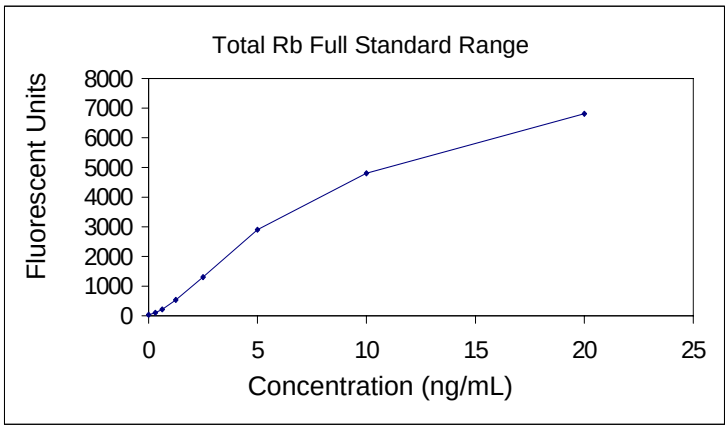
****Important note: The concentration of reconstituted standard is lot-specific. Please verify all concentration values entered in data analysis software.**

Reconstitution: Reconstitute in 3.0 mL *Assay Diluent*.

Recommended Starting Concentration for Standard Curve: Upon reconstitution, the starting concentration of the standard is the value cited above. Make serial 1:2 dilutions in *Assay Diluent*. Use 100 µL per assay.

Performance Characteristics

Analytical Sensitivity: The analytical sensitivity of the total Rb assay is <100 pg/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 Rb extractable from approximately 3 x 10^4 Jurkat cells using NP40 Cell Lysis Buffer (formulation presented below). The assay was found to be at least twice as sensitive as Western blotting.



Representative Standard Curve

Specificity: This kit is specific for Rb, independent of its phosphorylation state and does not display any cross-reactivity with AKT, JNK1/2, p38 MAPK, or Tau. Solutions of a panel of diluted cell extracts at 100 µg/mL or 400 µg/mL were assayed with the Invitrogen Human Total Rb Antibody Bead Kit. This assay reacts with Rb in both phosphorylated and unphosphorylated forms and equal levels of Rb are measured with this kit in lysates of Jurkat cells +/- staurosporine (a known inhibitor of Rb phosphorylation). The assay does not detect Rb in lysates from SaoS2, a cell line negative for Rb, while measuring significant levels of Rb in lysates from Jurkat, CRF-CEM, Colo305, U20S and HeLa cell lines.

Precision:

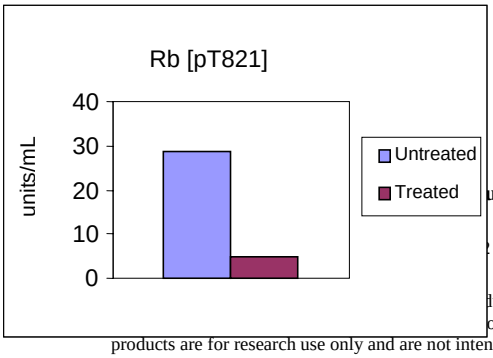
	Intra-assay	**Inter-assay**
Mean (pg/mL)	2500	2580
SD	202	253
%CV	8.1	9.8

Linearity of Dilution: Cell Extract buffer was spiked with human Total Rb and serially diluted in Assay Diluent, over the range of the assay. Linear regression analysis of samples versus the expected concentration yielded a correlation coefficient of 0.97.

Recovery: Cell lysates prepared in Extraction Buffer (diluted 1:20 in Assay Diluent) averaged 92% (range: 80% to 98%). Tissue culture medium containing 10% fetal calf serum averaged 100% (range: 95% to 105%).

To further evaluate the performance of this kit, a study using staurosporine was undertaken. In this study, Jurkat cells grown in RPMI medium containing 10% FBS were either left untreated, or treated with 100 nM staurosporine for 30 hours at 37°C, and the levels of Rb [pT821] (Figure A) and total Rb (Figure B) were determined. This study indicated that the level of Rb remained approximately constant, while the phosphorylation state of Rb decreased with staurosporine treatment. The data presented in Figure C show the results of normalizing the level of Rb [pT821] to total Rb.

A



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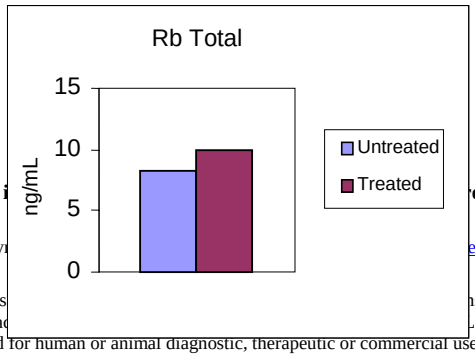

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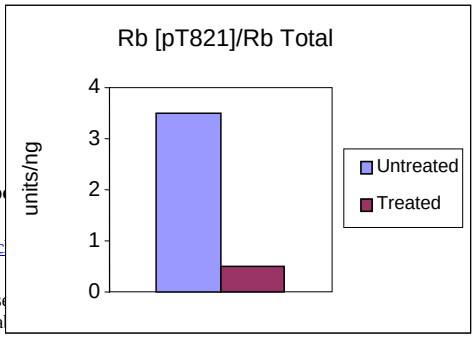
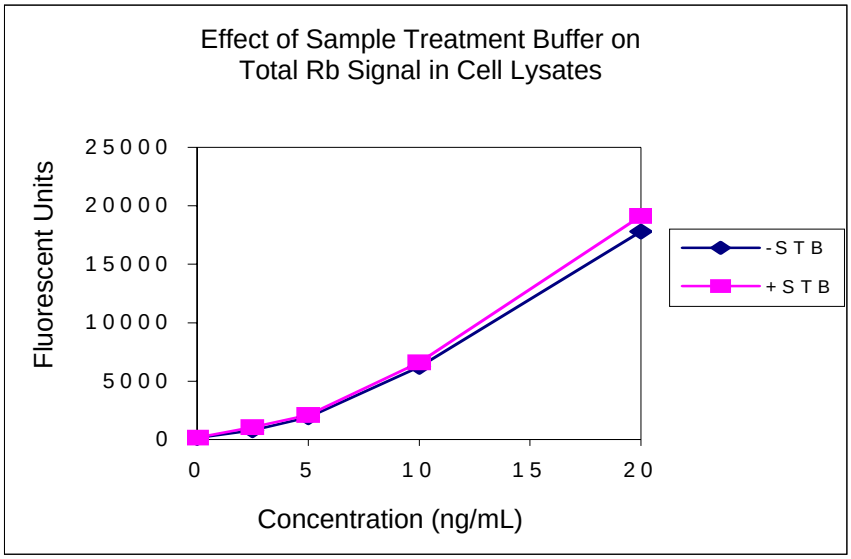


Sample Preparation:

This kit has been validated with cell lysates prepared in NP40 Cell Lysis Buffer (Invitrogen Cat. # FNN0021; 50 mM Tris, pH 7.4, 250 mM NaCl, 5 mM EDTA, 50 mM NaF, 1 mM Na_3VO_4, 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 10^8 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 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 impact of the *Sample Treatment Buffer* pre-incubation step on the signal observed with the Total Rb kit. In this study, Jurkat cells were lysed in NP40 Cell Lysis Buffer at a concentration of 1 x 10^8 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).



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