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Data Sheet

TYR Phosphorylation Assay Kit

Catalog #79502
Size: 96 reactions

DESCRIPTION: The *Tyrosine Phosphorylation Assay Kit* is designed to measure TYR phosphorylation of proteins. The *Tyrosine Phosphorylation Assay Kit* comes in a convenient format, with a 96-well plate, primary antibody against phosphorylated TYR residues, secondary HRP-labeled antibody, assay buffer, and phosphorylated standard for 100 enzyme reactions. The key to the *Tyrosine Phosphorylation Assay Kit* is a specific antibody mixture that provides sequence-independent recognition of phosphor-TYR residues. With this kit, only three simple steps on a microtiter plate are required for detection of the phosphorylation state. First, the protein of interest is coated on the strip plate. Next, primary antibody is added. Finally, the strips are treated with an HRP-labeled secondary antibody followed by addition of the HRP substrate to produce chemiluminescence that can then be measured using a chemiluminescence reader.

COMPONENTS:

Catalog #	Component	Amount	Storage	
52140Z5	Primary Antibody 30	12.5 µl	-80°C	***Avoid freeze/ thaw cycles!***
52130H	Secondary HRP-labeled Antibody 1	10 µl	-80°C	
	1x Phosphorylation Buffer	3 x 1 ml	-20°C	
79556	Blocking Buffer	50 ml	+4°C	
	Phosphorylated JAK2 (standard)	50 µl	-80°C	
	HRP chemiluminescent substrate A (translucent bottle)	6 ml	+4°C	
	HRP chemiluminescent substrate B (brown bottle)	6 ml	+4°C	
	96-well strip plate	1 plate	RT	

MATERIALS OR INSTRUMENTS REQUIRED BUT NOT SUPPLIED:
Protein of interest
TBST buffer (1x Tris-buffered saline, pH 8.0, containing 0.05% Tween-20)
Luminometer or fluorescent microplate reader capable of reading chemiluminescence
Rotating or rocker platform.
Adjustable micropipettor and sterile tips

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APPLICATIONS: Great for detection of tyrosine phosphorylation of recombinant proteins. Useful to examine the change of tyrosine phosphorylation level in response to various treatments.

STABILITY: One year from date of receipt when stored as directed.

REFERENCE(S): Wang, J.Y.J. 1988. Antibodies for phosphotyrosine: Analytical and preparative tool for tyrosyl-phosphorylated proteins. *Anal. Biochem.***172(1):** 1-7.

ASSAY PROTOCOL:
All samples and controls should be tested in duplicate.

Step 1:

1) Rehydrate the microwells by adding 150 µl of TBST buffer (1x TBS, pH 8.0, containing 0.05% Tween-20) to every well. Incubate 15 minutes at room temperature. Tap the strips onto clean paper towels to remove liquid.

2) Thaw your protein of interest and the provided **Phosphorylated JAK2 (standard)** on ice. Dilute **Phosphorylated JAK2 (standard)** to 10 ng/µl. Prepare several dilutions using **1x Phosphorylation Buffer.**

3) Add 50 µl per well of diluted protein of interest and **Phosphorylated JAK2 (standard)**.

4) Incubate at room temperature for 1 hour with slow shaking.

5) Wash the strips three times with 100 µl of TBST buffer. Blot dry with paper towels.

6) Add 100 µl of **Blocking Buffer** to every well. Shake on a rotating platform for 10 min. Remove supernatant as described above.

Step 2:

1) Dilute **Primary antibody 30** 800-fold with **Blocking Buffer**.

2) Add 100 µl per well. Incubate 1 hour at room temperature with slow shaking.

3) Wash the strips three times with 100 µl of TBST buffer. Blot dry onto clean paper towels.

4) Add 100 µl of **Blocking Buffer** to every well. Shake on a rotating platform for 10 min. Remove supernatant as described above.

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Step 3:

1) Dilute **Secondary HRP-labeled antibody 2** 1,000-fold with **Blocking Buffer**.

2) Add 100 µl per well. Incubate for 30 min. at room temperature with slow shaking.

3) Wash the strips three times with 100 µl of TBST buffer. Blot dry onto clean paper towels.

4) Add 100 µl of **Blocking Buffer** to every well. Shake on a rotating platform for 10 min. Remove supernatant as described above.

5) Just before use, mix on ice 50 µl **HRP chemiluminescent substrate A** and 50 µl **HRP chemiluminescent substrate B** and add 100 µl per well. Discard any unused chemiluminescent reagent after use.

6) Immediately read sample in a luminometer or microtiter-plate capable of reading chemiluminescence. “Blank” value is subtracted from all readings.

Reading Chemiluminescence:

Chemiluminescence is the emission of light (luminescence) which results from a chemical reaction. The detection of chemiluminescence requires no wavelength selection because the method used is emission photometry and is not emission spectrophotometry.

To properly read chemiluminescence, make sure the plate reader is set for LUMINESCENCE mode. Typical integration time is 1 second; delay after plate movement is 100 msec. Do not use a filter when measuring light emission. Typical settings for the Synergy 2 BioTek plate reader are: use the “hole” position on the filter wheel; Optics position: Top; Read type: endpoint. Sensitivity may be adjusted based on the luminescence of a control assay without enzyme (typically we set this value as 100).

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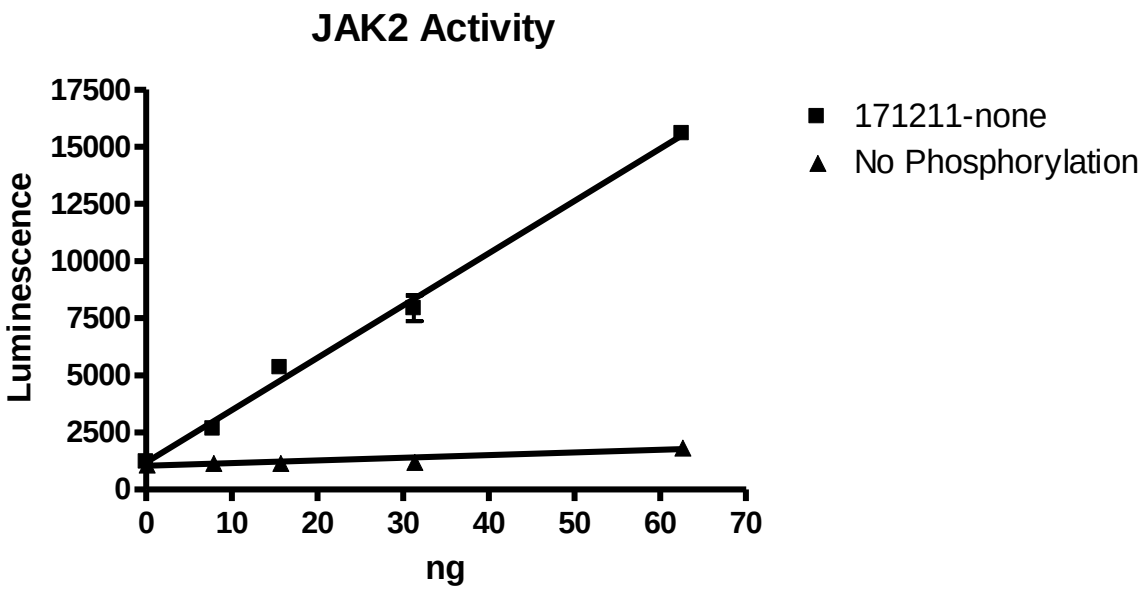

Example of Assay Results:

TYR phosphorylation of JAK2 measured using the *Tyrosine Phosphorylation Assay Kit*, BPS Bioscience #79502 Luminescence was measured using a Bio-Tek fluorescent microplate reader.

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RELATED PRODUCTS

Product Name	Catalog #	Size
Jak2 (JH1 domain), His-tag	40450	10 µg
Jak2 (JH1, JH2 domain), His-GST-tags	40451	10 µg
Kinase Buffer 1	79334	10 ml
EGFR Kinase Assay Kit	40321	96 rxns.
EGFR(L858R) Kinase Assay Kit	40324	96 rxns.
EGFR (T790M/C797S/ L858) Kinase Assay Kit	40326	96 rxns.
B-Raf(V600E) Kinase Assay Kit	48688	96 rxns.

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