Description

The TYR Phosphorylation Assay Kit is designed for measuring TYR (tyrosine) phosphorylation and screening and profiling small molecules that affect it. This kit comes in a convenient 96-well format, with enough antibody specific for phosphorylated TYR residues, HRP-labeled antibody and assay buffer for 100 reactions. This kit also includes phosphorylated JAK2 (Janus kinase 2) as standard control.

Background

TYR (tyrosine) phosphorylation is a modification of proteins where a phosphate group is added to the amino acid tyrosine by tyrosine kinases. This modification is crucial to regulate enzyme activity and signaling pathways, such as the Ras-MAPK (mitogen activated protein kinase) signaling pathway, and is needed for proteins involved in cell differentiation, cell cycle, gene regulation and transcription, angiogenesis and many other functions. Tyrosine kinases can be receptor or non-receptor, with RTK (receptor tyrosine kinases) being transmembrane proteins that can bind ligands extracellularly, while NRTK are mostly soluble intracellular proteins. Tyrosine phosphorylation can be reverted by PTPs (tyrosine phosphatases). Abnormal tyrosine phosphorylation is linked to several diseases, such as cancer and diabetes. An understanding of the TYR phosphorylation state of a protein and how to manipulate it, via the partners involved, is critical for the development of targeted therapies for tyrosine phosphorylation linked diseases.

Applications

- Detect TYR phosphorylation in recombinant proteins.
- Screening and profiling of small molecules that can alter TYR phosphorylation.

Supplied Materials

Catalog #	Name	Amount	Storage
52140Z5	Primary Antibody 30	12.5 µl	-80°C
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*	50 µl	-80°C
	HRP Chemiluminescence Substrate A (translucent bottle)	6 ml	+4°C
	HRP Chemiluminescence Substrate B (brown bottle)	6 ml	+4°C
	96-well strip plate (coated)	1 plate	Room Temp

** The initial concentration of enzyme is lot-specific and will be indicated on the tube containing the protein.*

Materials Required but Not Supplied

- Recombinant protein of interest
- TBST Buffer (1x Tris-buffered saline, pH 8.0, containing 0.05% Tween-20)
- Luminometer or microplate reader capable of reading chemiluminescence.
- Adjustable micropipettor and sterile tips
- Rotating or rocker platform

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Storage Conditions

This assay kit will perform optimally for up to **6 months** from date of receipt when the materials are stored as directed.

Safety

This product is for research purposes only and not for human or therapeutic use. This product should be considered hazardous and is harmful by inhalation, in contact with skin, eyes, clothing, and if swallowed. If contact occurs, wash thoroughly.

Contraindications

The final concentration of DMSO in the assay should not exceed 1%.

Assay Protocol

- All samples and controls should be performed in duplicate.
- The assay should include "Blank", "Standard" and "Test" conditions.
- We recommend maintaining the diluted proteins on ice during use.
- For detailed information on protein handling please refer to Protein FAQs (bpsbioscience.com).
- We recommend using phosphorylated JAK2 as internal control. If not running a dose response curve for the control inhibitor, we recommend running the control inhibitor at 0.1X, 1X and 10X the IC_{50} value shown in the validation data below.

Step 1

1. Add 150 µl of TBST Buffer to the wells in order to rehydrate them.
2. Incubate 15 minutes at Room Temperature (RT).
3. Tap the plate onto clean paper towel to remove the liquid.
4. Thaw **protein of interest** and **phosphorylated JAK2** on ice. Briefly spin the tube to recover the full content.
5. Dilute phosphorylated JAK2 to 10 ng/µl with 1x Phosphorylation Buffer.
6. Prepare a serial dilution of phosphorylated JAK2 in 1x Phosphorylation Buffer, with 10 ng/µl being the top value of the dilution (50 µl/well).
7. Dilute protein of interest to the appropriate value using 1x Phosphorylation Buffer.
8. Prepare a serial dilution of the protein of interest in 1x Phosphorylation Buffer (50 µl/well).
9. Add 50 µl of diluted phosphorylated JAK2 to the "Standard" wells.
10. Add 50 µl of the diluted protein of interest to the "Test" wells.
11. Add 50 µl of 1x Phosphorylation Buffer to the "Blank" wells.

12. Incubate for 1 hour at RT with gentle agitation.
13. Wash the wells three times with 100 µl of TBST Buffer.
14. Tap the plate onto clean paper towel to remove the liquid.
15. Add 100 µl of Blocking Buffer to all wells.
16. Agitate for 10 minutes at RT.
17. Tap the plate onto clean paper towel to remove the liquid.

Step 2:

1. Dilute 800-fold the Primary Antibody 30 with Blocking Buffer.
2. Add 100 µl of diluted Primary Antibody 30 to each well.
3. Incubate 1 hour at RT with gentle agitation.
4. Wash the wells three times with 100 µl of TBST Buffer.
5. Tap the plate onto clean paper towel to remove the liquid.
6. Add 100 µl of Blocking Buffer to all wells.
7. Agitate for 10 minutes at RT.
8. Tap the plate onto clean paper towel to remove the liquid.

Step 3:

1. Dilute 1,000-fold the Secondary HRP-Labeled Antibody 1 with Blocking Buffer.
2. Add 100 µl of diluted Secondary HRP-Labeled Antibody 1 to each well.
3. Incubate 30 minutes at RT with gentle agitation.
4. Wash the wells three times with 100 µl of TBST Buffer.
5. Tap the plate onto clean paper towel to remove the liquid.
6. Add 100 µl of Blocking Buffer to all wells.
7. Agitate for 10 minutes at RT.
8. Tap the plate onto clean paper towel to remove the liquid.

9. Prepare a Mix (100 µl/well): N wells x (50 µl of HRP Chemiluminescence Substrate A + 50 µl of HRP Chemiluminescence Substrate B).

10. Add 100 µl of Mix to each well.

11. Immediately ready samples in a luminometer or microplate reader capable of reading chemiluminescence.

12. The "Blank" value should be subtracted from all other values.

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)

Example Results

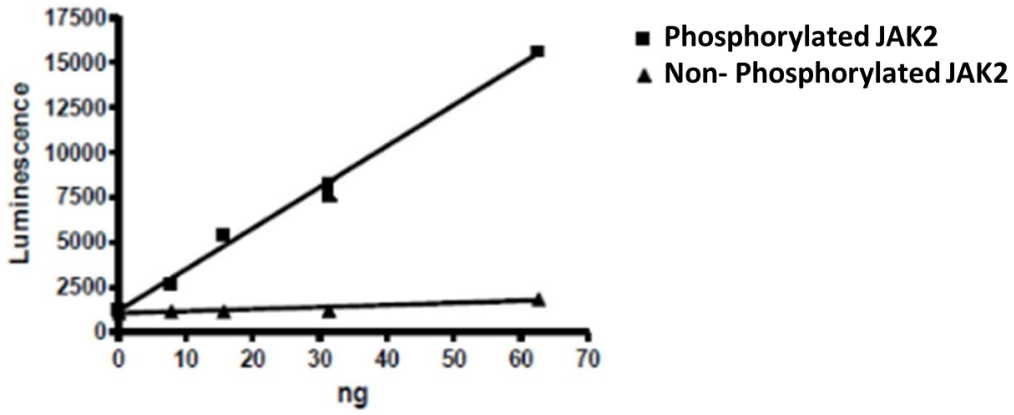

Figure 2: TYR phosphorylation analysis of JAK2.
Phosphorylation was measured in phosphorylated JAK2 and non-phosphorylated JAK2 at different concentrations. Luminescence was measured using a Bio-Tek fluorescent microplate reader.

Data shown is representative. For lot-specific information, please contact BPS Bioscience, Inc. at support@bpsbioscience.com.

Troubleshooting Guide

Visit bpsbioscience.com/assay-kits-faq for detailed troubleshooting instructions. For further questions, please email support@bpsbioscience.com

References

Hunter T., 2009 *Curr Opin Cell Biol* 21(2):140-6.

Related Products

Products	*Catalog #*	*Size*
JAK1 (Janus Kinase 1) Assay Kit	79518	96 reactions
JAK2 (Janus Kinase 2) Assay kit	79520	96 reactions
JAK3 (Janus Kinase 3) Assay Kit	79521	96 reactions
TYK (Tyrosine Kinase 2) Assay Kit	79075	96 reactions

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