Revision 12

Store at +4C

#88918

PathScan® RP Phospho-RIP (Ser166) Chemiluminescent Sandwich ELISA Kit

Cell Signaling TECHNOLOGY®

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1 Kit (96 assays)

Species Cross Reactivity: H M

UniProt ID: #Q13546

Entrez-Gene Id: #8737

For Research Use Only. Not for Use in Diagnostic Procedures.

Product Includes	Product #	Quantity	Color	Storage Temp
RIP Rabbit mAb Coated Microwells	94588	96 tests		+4C
Phospho-RIP (Ser166) Rabbit Detection mAb	24352	1 ea	Red (Lyophilized)	+4C
HRP Diluent	13515	5.5 ml	Red	+4C
Luminol/Enhancer Solution	84850	3 ml		RT
Stable Peroxide Buffer	42552	3 ml		RT
Sealing Tape	54503	2 ea		+4C
ELISA Wash Buffer (20X)	9801	25 ml		+4C
Cell Lysis Buffer (10X)	9803	15 ml		-20C

Kit contents scale proportionally with size, except sealing tape.
Example: The V1 kit contains 5X the listed quantities above, but will exclude the sealing tape.

The microwell plate is supplied as 12 8-well modules - Each module is designed to break apart for 8 tests.

Description

The rapid protocol (RP) PathScan® RP Phospho-RIP (Ser166) Chemiluminescent Sandwich ELISA Kit is a solid phase sandwich enzyme-linked immunosorbent assay (ELISA) that detects endogenous levels of RIP protein phosphorylated at Ser166 in a reduced assay time of 1.5 hours. This chemiluminescent ELISA, which is offered in low volume microplates, shows increased signal and sensitivity while using a smaller sample size. Incubation of cell lysates and detection antibody on the coated microwell plate forms a sandwich with phospho-RIP (Ser166) protein in a single step. The plate is then extensively washed and chemiluminescent reagent is added for signal development. The magnitude of light emission, measured in relative light units (RLU), is proportional to the quantity of RIP protein phosphorylated at Ser166. Learn more about all of your ELISA kit options here.

*Antibodies in this kit are custom formulations specific to kit.

Specificity/Sensitivity

The PathScan® RP Phospho-RIP (Ser166) Chemiluminescent Sandwich ELISA Kit detects endogenous levels of RIP protein phosphorylated at Ser166. The kit sensitivity is shown in Figure 1. This kit detects proteins from the indicated species, as determined through in-house testing, but may also detect homologous proteins from other species.

Background

The receptor-interacting protein (RIP) family of serine-threonine kinases (RIP, RIP2, RIP3, and RIP4) are important regulators of cellular stress that trigger pro-survival and inflammatory responses through the activation of NF-κB, as well as pro-apoptotic pathways (1). In addition to the kinase domain, RIP contains a death domain responsible for interaction with the death domain receptor Fas and recruitment to TNF-R1 through interaction with TRADD (2,3). RIP-deficient cells show a failure in TNF-mediated NF-κB activation, making the cells more sensitive to apoptosis (4,5). RIP also interacts with TNF-receptor-associated factors (TRAFs) and can recruit IKKs to the TNF-R1 signaling complex via interaction with NEMO, leading to IκB phosphorylation and degradation (6,7). Overexpression of RIP induces both NF-κB activation and apoptosis (2,3). Caspase-8-dependent cleavage of the RIP death domain can trigger the apoptotic activity of RIP (8).

Necroptosis, a regulated pathway for necrotic cell death, is triggered by a number of inflammatory signals including cytokines in the tumor necrosis factor (TNF) family, pathogen sensors such as toll-like receptors (TLRs), and ischemic injury (9,10). The process is negatively regulated by caspases and is initiated through a complex containing the RIP and RIP3 kinases, typically referred to as the necrosome. Necroptosis is inhibited by a small molecule inhibitor of RIP, necrostatin-1 (Nec-1) (11). Research studies show that necroptosis contributes to a number of pathological conditions, and Nec-1 has been shown to provide neuroprotection in models such as ischemic brain injury (12). RIP is phosphorylated at several sites within the kinase domain that are sensitive to Nec-1, including Ser14, Ser15, Ser161, and Ser166 (13).

Background References

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2. Hsu, H. et al. (1996) *Immunity* 4, 387-96.
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Limited Uses

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PathScan® Chemiluminescent Sandwich ELISA Protocol (Rapid Protocol)

NOTE: This protocol is for PathScan® kits that use an HRP directly conjugated to the detection antibody **(Rapid Protocol)**, rather than a 2-step method where the detection antibody and a secondary-HRP are added sequentially.

This chemiluminescent ELISA is offered in low volume microplates. Only a total volume of 50 µL (samples and reagents) are required in each microwell.

A. Solutions and Reagents

NOTE: Prepare solutions with deionized/purified water or equivalent.

1. **Microwell strips:** Bring all to room temperature before opening bag/use. Unused microwell strips should be returned to the original re-sealable bag containing the desiccant pack and stored at 4°C.
2. **Detection Antibody:** Reconstitute lyophilized Detection Antibody (red colored cake) with 3 mL HRP Diluent. Incubate at room temperature for 5 min with occasional gentle mixing to fully reconstitute. For best results, use immediately following antibody reconstitution. Unused reconstituted Detection Antibody may be stored for up to 4 weeks at 4°C, although there may be some loss of signal compared to freshly reconstituted antibody.
3. **HRP Diluent:** Red colored diluent for reconstitution and dilution of the Detection Antibody that is linked to HRP.
4. **1X ELISA Wash Buffer:** Prepare by diluting ELISA Wash Buffer (20X) (included in each kit) to 1X with deionized water.
5. **1X Cell Lysis Buffer:** Prepare by diluting 10X Cell Lysis Buffer #9803 to 1X with deionized water. This buffer can be stored at 4°C for short-term use (1–2 weeks). Recommended: When using to prepare cell lysates, add Protease/Phosphatase Inhibitor Cocktail (#5872, not supplied) and 1 mM phenylmethyl- sulfonyl fluoride (PMSF, #8553, not supplied) immediately before use.
6. **Luminol/Enhancer Solution** and **Peroxide Buffer**

B. Preparing Cell Lysates

For adherent cells

1. Aspirate media when the culture reaches 80–90% confluence. Treat cells by adding fresh media containing regulator for desired time.
2. Remove media and rinse cells once with ice-cold 1X PBS.
3. Remove PBS and add 0.5 mL ice-cold 1X Cell Lysis Buffer including 1 mM PMSF and Protease/Phosphatase Inhibitor Cocktail to each plate (10 cm diameter) and incubate the plate on ice for 5 min.
4. Scrape cells off the plate and transfer to an appropriate tube. Keep on ice.
5. Sonicate lysates on ice.
6. Microcentrifuge for 10 min (14,000 rpm) at 4°C and transfer the supernatant to a new tube. The supernatant is the cell lysate. Store at −80°C in single-use aliquots.

For suspension cells

1. Remove media by low speed centrifugation (~1200 rpm) when the culture reaches 0.5–1.0 x 10^6 viable cells/mL. Treat cells by adding fresh media containing regulator for desired time.
2. Collect cells by low speed centrifugation (~1200 rpm) and wash once with 5-10 mL ice-cold 1X PBS.
3. Cells harvested from 50 mL of growth media can be lysed in 2.0 mL of 1X Cell Lysis Buffer including 1 mM PMSF and Protease/Phosphatase Inhibitor Cocktail.
4. Sonicate lysates on ice.
5. Microcentrifuge for 10 min (14,000 rpm) at 4°C and transfer the supernatant to a new tube. The supernatant is the cell lysate. Store at −80°C in single-use aliquots.

C. Test Procedure

NOTE: Equilibrate all materials and prepared reagents to room temperature prior to running the assay.

1. Prepare all reagents as indicated above (Section A).
2. Samples should be undiluted or diluted with 1X Cell Lysis Buffer to a 2X protein concentration in order to achieve a final 1X protein concentration upon addition of the Detection Antibody. Individual datasheets for each kit provide a sensitivity curve that serves as a reference for selection of an appropriate starting lysate concentration. The sensitivity curve shows typical results across a range of lysate concentration points.
3. Add 25 µL of each sample to the appropriate wells.
4. Add 25 µL of the Detection Antibody to each well.

5. Seal the plate and incubate for 1 hour at room temperature on a plate shaker set to 400 rpm (moderate agitation).
6. Gently remove the tape and wash wells:
    1. Discard plate contents into a receptacle.
    2. Wash 4 times with 1X Wash Buffer, 150 µL each time for each well.
    3. For each wash, strike plates on fresh towels hard enough to remove the residual solution in each well, but do not allow wells to completely dry at any time.
    4. Clean the underside of all wells with a lint-free tissue.
7. Prepare Detection Reagent Working Solution by mixing equal parts Luminol/Enhancer Solution and Stable Peroxide Buffer.
8. Add 50 µL of the Detection Reagent Working Solution to each well.
9. Use a plate-based luminometer to measure Relative Light Units (RLU) at 425 nm within 1–10 min following addition of the substrate. Optimal signal intensity is achieved when read within 10 min.