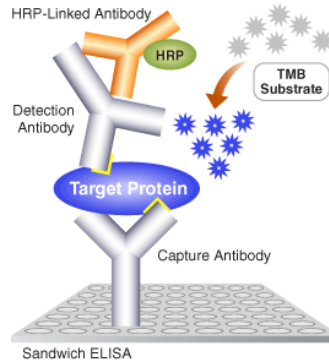


## Cell Signaling has the PathScan ELISA Antibody Pairs

- Enable the detection of low amounts of target protein from cell lysates
- Matched modification state and total ELISA kits available
- Multitarget kits available for parallel analysis of several signaling molecules

### Kit Components

1. Antibody coated microplates
2. Detection antibody (green)
3. HRP-linked antibody
4. 20X Wash Buffer
5. 10X Cell lysis buffer
6. Sample Diluent Buffer (blue)
7. TMB Substrate
8. Stop Solution



### Reagent Preparation

1. **Coating Buffer:** 1X PBS, ([20X PBS #9808](#))  
3.2 mM Na<sub>2</sub>HPO<sub>4</sub>, 0.5 mM KH<sub>2</sub>PO<sub>4</sub>, 1.3 mM KCl, 135 mM NaCl, pH 7.4
2. **Wash Buffer:** 1X PBS/0.05% Tween-20, ([20X PBST #9809](#))
3. **Blocking Buffer:** 1X PBS/0.05% Tween-20, 1% BSA
4. **1X Cell Lysis Buffer:** ([10X Cell Lysis Buffer #9803](#))  
20 mM Tris (pH 7.5), 150 mM NaCl, 1 mM ethylene diamine tetraacetate (EDTA),  
1 mM ethylene glycol-bis(2-aminoethyl)-N,N,N',N'-tetraacetic acid (EGTA),  
1% Triton X-100, 2.5 mM sodium pyrophosphate, 1 mM β-glycerophosphate,  
1 mM Na<sub>3</sub>VO<sub>4</sub>, 1 μg/ml leupeptin.
5. **TMB Substrate:** ([TMB Substrate #7004](#))
6. **STOP Solution:** ([STOP Solution #7002](#))
7. Dilute detection antibody 1:100 in Blocking Buffer. For a single 96 well plate, add 100 μl of Detector Antibody Stock to 9.9 ml of Blocking Buffer
8. Secondary antibody, either, anti-mouse or anti-rabbit-HRP, is diluted 1:1000 in Blocking Buffer. For a single 96 well plate, add 10 μl of secondary antibody stock to 9.99 ml of Blocking Buffer

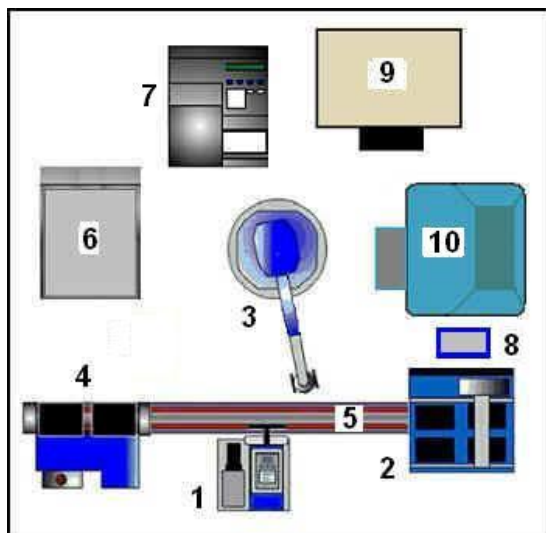
**NOTE:** Reagents should be made fresh daily

## Preparation of Cell Lysates

1. Aspirate media. Treat cells by adding fresh media containing regulator for desired time.
2. Remove media and rinse cells once with ice-cold PBS.
3. Remove PBS and add 0.5 ml ice-cold 1X Cell Lysis Buffer plus 1 mM phenylmethylsulfonyl fluoride (PMSF) to each plate (10 cm in diameter) and incubate the plate on ice for 5 minutes.
4. Scrape cells off the plate and transfer to an appropriate tube. Keep on ice.
5. Sonicate lysates on ice.
6. Microcentrifuge for 10 minutes 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.

## Coating Procedure

1. Rinse microplate with dH<sub>2</sub>O. Add 200 µl of dH<sub>2</sub>O and discard liquid. Blot on paper towel to make sure wells are dry.
2. Dilute capture antibody 1:100 in PBS. For a single 96 well plate, add 100 µl of Capture Antibody Stock to 9.9 ml PBS. Mix well and add 100 µl/well. Cover plate and incubate overnight at 4°C (17–20 hours).
3. **After overnight coating, gently uncover plate and wash wells:**
  1. Discard plate contents into a receptacle.
  2. Wash 4 times with Wash Buffer, 200 µl each time for each well. 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.
  3. Clean the underside of all wells with a lint-free tissue.
4. Block plates. Add 150 µl of Blocking Buffer/well, cover plate and incubate at 37°C for 2 hours.
5. After blocking, wash plate as in Step 3. Plate is ready to use.



1. Micro10 12-Channel Dispenser
2. SOLO Single Channel Pipettor
3. PlateCrane EX
4. StackLink
5. TrackLink
6. Synergy 4 Multi-Mode Reader
7. ELx405 Washer
8. TeleShake Microplate Agitator
9. SX40T Incubator

## Test Procedure



Micro10

**Dispense** 100  $\mu$ l of cell lysate per well and cover plates.



STX-40

**Incubate** at 37°C for 2 hours.



ELx405

**Wash** Wash 4 times with Wash Buffer, 200  $\mu$ l each time for each well. 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.



Micro10

**Dispense** 100  $\mu$ l of detector antibody/blocking buffer mixture per well and cover plates



STX-40

**Incubate** at 37°C for 1 hour.



ELx405

**Wash** Wash 4 times with Wash Buffer, 200  $\mu$ l each time for each well. 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.



Micro10

**Dispense** 100  $\mu$ l of secondary antibody per well and cover plate.



STX-40

**Incubate** at 37°C for 30 minutes.



ELx405

**Wash** Wash 4 times with Wash Buffer, 200  $\mu$ l each time for each well. 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.



Micro10

**Dispense** 100  $\mu$ l of TMB Substrate per well and cover plate.



STX-40

**Incubate** at 37°C for 10 minutes.



Micro10

**Dispense** 100  $\mu$ l of STOP Solution per well



Synergy 4

**Read** plate on a microplate reader at Absorbance 450 nm.