

# PathScan® Phospho-IκB-α (Ser32) Sandwich ELISA Antibody Pair

✓ 1 Kit  
(4 X 96 assays)

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This product is for *in vitro* research use only and is not intended for use in humans or animals.  
This product is not intended for use as a therapeutic or in diagnostic procedures.

Entrez-Gene ID #4792  
Swiss-Prot Acc. #P25963

## Species Cross-Reactivity: H, M

**Description:** CST's PathScan® Phospho-IκB-α (Ser32) Sandwich ELISA Antibody Pair is being offered as an economical alternative to our PathScan® Phospho-IκB-α (Ser32) Sandwich ELISA Kit #7355. Capture and Detection antibodies (100X stocks) and HRP-conjugated secondary antibody (1000X stock) are supplied. Sufficient reagents are supplied for 4 x 96 well ELISAs. The IκB-α Capture Antibody is coated in PBS overnight in a 96 well microplate. After blocking, cell lysates are added followed by a Phospho-IκB-α (Ser32) Detection Antibody and anti-Rabbit IgG, HRP conjugated antibody. HRP substrate, TMB, is added for color development. The magnitude of the absorbance for this developed color is proportional to the quantity of Phospho-IκB-α (Ser32) protein.

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

### Reagents not supplied:

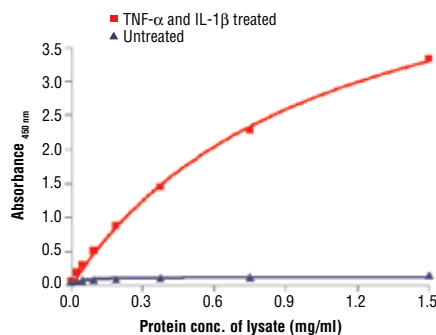
Phosphate Buffered Saline (PBS-20X) #9808  
Phosphate Buffered Saline with Tween-20 (PBST-20X) #9809  
Cell Lysis Buffer (10X) #9803  
TMB Substrate #7004  
STOP Solution #7002  
Blocking Buffer: 1X PBS/0.05% Tween-20, 1% BSA  
96 Well Microplates\*\*  
Microplate Reader

\*\* Antibody Pairs have been validated on Corning® 96 Well Clear Polystyrene High Bind Stripwell™ Microplates (#2592) and Corning® 96 Well EIA/RIA Easy Wash™ Clear Flat Bottom Polystyrene High Bind Microplates (#3369).

**Notes:** Antibody pairs have been optimized using recommended buffers, reagents, plates and the included protocol. Solutions should be made fresh daily.

**Background:** The NF-κB/Rel transcription factors are present in the cytosol in an inactive state complexed with the inhibitory IκB proteins (1-3). Activation occurs via phosphorylation of IκB-α at Ser32 and Ser36 followed by proteasome-mediated degradation that results in the release and nuclear translocation of active NF-κB (3-7). IκB-α phosphorylation and resulting Rel-dependent transcription are activated by a highly diverse group of extracellular signals including inflammatory cytokines, growth factors and chemokines. Kinases that phosphorylate IκB at these activating sites have been identified (8). Because phos-

Products Included	Volume	Cap Color	Storage
IκB-α Mouse Capture Antibody (100X)	0.4 ml	Pink	4°C
Phospho-IκB-α (Ser32) Rabbit Detection Antibody (100X)	0.4 ml	Blue	4°C
Anti-rabbit IgG, HRP-Linked Antibody (1000X)	0.04 ml	Red	-20°C



The relationship between lysate protein concentration from untreated and TNF-α and IL-1β treated HeLa cells and the absorbance at 450 nm using PathScan® Phospho-IκB-α (Ser32) Sandwich ELISA Antibody Pair #7343 is shown. HeLa cells were treated with TNF-α and IL-1β for 5 minutes at 37°C and then lysed.

phorylation of IκB-α at Ser32/36 is essential for release of active NF-κB, phosphorylation at this site is an excellent marker of NF-κB activation (1-3).

### Background References:

- (1) Baeuerle, P.A. and Baltimore, D. (1988) *Science* 242, 540-6.
- (2) Beg, A.A. and Baldwin, A.S. (1993) *Genes Dev* 7, 2064-70.
- (3) Finco, T.S. et al. (1994) *Proc Natl Acad Sci USA* 91, 11884-8.
- (4) Brown, K. et al. (1995) *Science* 267, 1485-8.
- (5) Brockman, J.A. et al. (1995) *Mol Cell Biol* 15, 2809-18.
- (6) Traenckner, E.B. et al. (1995) *EMBO J* 14, 2876-83.
- (7) Chen, Z.J. et al. (1996) *Cell* 84, 853-62.
- (8) Karin, M. and Ben-Neriah, Y. (2000) *Annu Rev Immunol* 18, 621-63.

**Storage:** Capture and Detection Antibodies are stored at 4°C. Anti-Rabbit IgG, HRP-Linked Antibody is stored at -20°C.

### Companion Products:

Phosphate Buffered Saline (PBS-20X) #9808  
Phosphate Buffered Saline with Tween 20 (PBST-20X) #9809  
BSA #9998  
TMB Substrate #7004  
STOP Solution #7002  
Anti-rabbit IgG, HRP-linked Antibody #7074  
PathScan® Phospho-IκB-α (Ser32) Sandwich ELISA Kit #7355  
PathScan® Total IκB-α Sandwich ELISA Kit #7360  
IκBα (44D4) Rabbit mAb #4812  
IκB-α (L35A5) Mouse mAb (Amino-terminal Antigen) #4814  
IκB-α (112B2) Mouse mAb (Carboxy-terminal Antigen) #9247  
IκB-α Antibody #9242  
Phospho-IκBα (Ser32) (14D4) Rabbit mAb #2859  
Phospho-IκBα (Ser32/36) (5A5) Mouse mAb #9246  
Phospho-IκB-α (Ser32/36) (12C2) Mouse mAb #5210  
PhosphoPlus® IκBα (Ser32/36) Antibody Kit #9240  
Phospho-IκB-α (Ser32) Antibody #9241  
Phospho-IκB-α (Ser32/36) (5A5) mAb #5205  
Cell Lysis Buffer (10X) #9803

## PathScan® Sandwich ELISA Antibody Pair Protocol

### A Required Reagents

- 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
- Wash Buffer:** 1X PBS/0.05% Tween-20, (20X PBST #9809)
- Blocking Buffer:** 1X PBS/0.05% Tween-20, 1% BSA
- 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.
- TMB Substrate:** (TMB Substrate #7004)
- STOP Solution:** (STOP Solution #7002)

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

### B Coating Procedure

- 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.
- 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).
- After overnight coating, gently uncover plate and wash wells:**
  - Discard plate contents into a receptacle.
  - 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.
  - Clean the underside of all wells with a lint-free tissue.
- Block plates. Add 150 μl of Blocking Buffer/well, cover plate and incubate at 37°C for 2 hours.
- After blocking, wash plate as in Step 3. Plate is ready to use.

### C Preparing Cell Lysates

- Aspirate media, treat cells by adding fresh media containing regulator for desired time.
- To harvest cells under nondenaturing conditions, remove media and rinse cells once with ice-cold PBS.
- Remove PBS and add 0.5 ml ice-cold 1X Cell Lysis Buffer plus 1 mM phenyl-methylsulfonyl fluoride (PMSF) to each plate (10 cm in diameter) and incubate the plate on ice for 5 minutes.
- Scrape cells off the plate and transfer to an appropriate tube. Keep on ice.
- Sonicate lysates on ice.
- 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.

### D Test Procedure

- Lysates can be used undiluted or diluted in Blocking Buffer. 100 μl of lysate is added per well. Cover plate and incubate at 37°C for 2 hours.
- Wash plate as in Coating Procedure, Step 3.
- 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. Mix well and add 100 μl/well. Cover plate and incubate at 37°C for 1 hour.
- Plate is washed as in Coating Procedure, Step 3.
- 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. Mix well and add 100 μl/well. Cover and incubate at 37°C for 30 minutes.
- Wash plate as in Coating Procedure, Step 3.
- Add 100 μl of TMB Substrate per well. Cover and incubate at 37°C for 10 minutes.
- Add 100 μl of STOP Solution per well.
- Read plate on a microplate reader at Absorbance 450 nm.