Store at -80°C

#8002

CD45 Phosphatase

☑5 µg

Cell Signaling TECHNOLOGY®

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New 03/08

Description: Purified recombinant human CD45 (Leu193-Ser1143) phosphatase, supplied as a GST fusion protein.

Background: The protein phosphatase (PTP) receptor CD45 is a type I transmembrane protein comprised of a pair of intracellular tyrosine phosphatase domains and a variable extracellular domain generated by alternative splicing (1). The catalytic activity of CD45 is a function of the first phosphatase domain (D1) while the second phosphatase domain (D2) may interact with and stabilize the first domain, or recruit/bind substrates (2,3). CD45 interacts directly with antigen receptor complex proteins or activates Src family kinases involved in the regulation of T- and B-cell antigen receptor signaling (1). Specifically, CD45 dephosphorylates Src-family kinases Lck and Fyn at their conserved negative regulatory carboxy-terminal tyrosine residues and upregulates kinase activity. Conversely, studies indicate that CD45 can also inhibit Lck and Fyn by dephosphorylating their positive regulatory autophosphorylation site. CD45 appears to be both a positive and a negative regulator that conducts signals depending on specific stimuli and cell type (1). Human leukocytes including lymphocytes, eosinophils, monocytes, basophils and neutrophils express CD45, while erythrocytes and platelets are negative for CD45 expression (4).

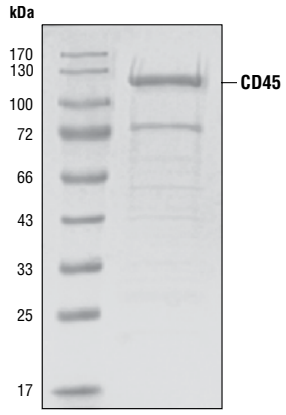

Figure 1. The purity of the GST-CD45 fusion protein was analyzed using SDS/PAGE followed by Coomassie stain.

Source/Purification: The GST-phosphatase fusion protein was produced by expressing recombinant human CD45 (Leu193-Ser1143)(GenBank Accession No. NM_080921) with an amino-terminal tail in E. coli. The protein was purified by one-step affinity chromatography using glutathione-agarose.

Quality Control: The theoretical molecular weight of the GST-CD45 fusion protein is 120 kDa. The purified kinase was quality controlled for purity using SDS-PAGE followed by Coomassie stain [Fig.1]. CD45 phosphatase activity was determined using a DELFIA® assay [Fig.2].

Background References:

(1) Huntington, N.D. and Tarlington, D.M. (2004) *Immunol. Lett.* 94, 167–174.

(2) Felberg, J. and Johnson, P. (2000) *Biochem. Biophys. Res. Commun.* 271, 292–298.

(3) Kashio, N. et al. (1998) *J. Biol. Chem.* 273, 33856–33863.

(4) Wang, Y. and Johnson, P. (2005) *J. Biol. Chem.* 280, 14318–14324.

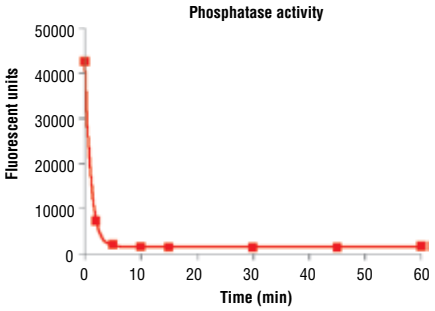

Figure 2. CD45 phosphatase activity was measured in a DELFIA® assay using the following reaction conditions: 25 mM HEPES, pH 7.2, 50 mM NaCl, 2.5 mM EDTA, 5 mM DTT, 65 ng/µl BSA, Substrate: Phospho-Poly (Glu-Tyr) Biotinylated Peptide #1586 at 1.5 µM, and 0.125 ng/µl CD45.

Entrez-Gene ID #5788
Swiss-Prot Acc. #P08575

Storage: Enzyme is supplied in 50 mM Tris-HCl, pH 7.5; 150 mM NaCl, 0.25 mM DTT, 0.1 mM EGTA, 0.1 mM EDTA, 0.1 mM PMSF, 25% glycerol, 7 mM glutathione.

Store at -80°C.

Keep on ice during use.

Avoid repeated freeze-thaw cycles.

Companion Products:

Poly (Glu-Tyr) Biotinylated Peptide #1585

Phospho-Poly (Glu-Tyr) Biotinylated Peptide #1586

Phospho-Tyrosine Mouse mAb (P-Tyr-100) #9411

Protocol for Tyrosine Phosphatase

Phosphatase Assay:

A Additional Solutions and Reagents (Not included)

- **Phosphatase Buffer (5X)**
  125 mM HEPES, pH 7.2
  250 mM NaCl
  12.5 mM EDTA
- Phospho-Poly (Glu-Tyr) Biotinylated Peptide #1586
- DTT (1.25 M)
- BSA (65 ng/µl)
- Stop solution (2N NaOH)
- Wash Buffer: 1X, PBS 0.05% Tween-20 (PBS/T)
- Phospho-Tyrosine mAb (P-Tyr-100) #9411

B Suggested Protocol for 100 Assays

1. Prepare fresh batches of 1X Phosphatase Assay Buffer by diluting the Phosphatase Buffer (5X) at a 1:4 ratio with a solution containing 5 mM DTT and 65 ng/µl BSA.
2. Dilute 1 mM Phospho-Poly EY (20) Biotinylated Peptide substrate solution to 3 µM with 1X Phosphatase Assay Buffer.
3. Thaw enzyme on ice.
4. Dilute phosphatase protein to 0.2 to 2.0 ng/µl with 1X Phosphatase Assay Buffer.
5. To start the reaction combine 25 µl diluted phosphatase solution and 25 µl substrate (3 µM). Incubate at 37°C for 5 to 60 minutes.

Final Assay Conditions for a 50 µl Reaction

25 mM HEPES, pH 7.2
50 mM NaCl
2.5 mM EDTA
5 mM DTT
65 ng/µl BSA
1.5 µM Phospho-Poly (Glu-Tyr) Biotinylated Peptide
0.1 to 1.0 ng/µl phosphatase

6. Terminate reaction by adding 50 µl of 2N NaOH Stop Solution to each reaction well.
7. For DELFIA® or colorimetric ELISA detection methods please use the protocols described to the right.

DELFIA® Assay

1. Prepare appropriate dilution of Europium labeled secondary antibody in PBS/T with 1% BSA (1:500 dilution for anti-mouse IgG or 1:1000 for anti-rabbit IgG).
2. Add 100 µl/well secondary antibody solution.
3. Incubate at room temperature for 30 minutes.
4. *Wash five times with 200 µl/well PBS/T.
5. Add 100 µl/well DELFIA® Enhancement Solution.
6. Incubate at room temperature for 5 minutes.
7. Read plate using a time resolved fluorescent plate reader using the following settings;
   a. Excitation Filter: 340 nm
   b. Emission Filter: 615 nm
   c. Delay++: 400 µs

++ Delay time is the delay from the excitation pulse to the beginning of the measurement.

Companion Products for DELFIA®

DELFIA® Europium-labeled Anti-mouse IgG (PerkinElmer Life Sciences #AD0124)
DELFIA® Europium-labeled Anti-rabbit IgG (PerkinElmer Life Sciences #AD0105)
DELFIA® Enhancement Solution (PerkinElmer Life Sciences #1244-105)
DELFIA® Streptavidin coated, 96-well, yellow plate (PerkinElmer Life Sciences AAAND-0005)

Colorimetric ELISA Assay

1. Prepare appropriate dilution of HRP labeled secondary antibody in PBS/T with 1% BSA (1:500 dilution for anti-mouse IgG or 1:1000 for anti-rabbit IgG).
2. Add 100 µl/well secondary antibody solution.
3. Incubate at room temperature for 30 minutes.
4. *Wash five times with 200 µl/well PBS/T.
5. Add 100 µl/well TMB substrate.
6. Incubate at room temperature for 15 minutes.
7. Add 100 µl/well of stop solution.
8. Mix well.
9. Read the absorbance at 450 nm with a microtiter plate reader.

Companion Products For Colorimetric ELISA Assay

Anti-mouse IgG, HRP Linked Antibody #7076
Anti-rabbit IgG, HRP Linked Antibody #7074
TMB Solution #7004
Stop Solution #7002

*** NOTE:** Use of an automated microplate washer as well as centrifugation of plates when appropriate, greatly improves reproducibility.

For any questions please contact:
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