

#3575 Store at -20°C

CD45 (136-4B5) Mouse mAb

400 µl
 (100 Tests)



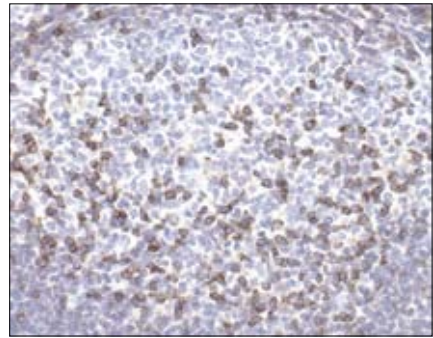
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This product is for *in vitro* research use only and is not intended for use in humans or animals.

Applications	Species Cross-Reactivity	Molecular Wt.	Source	Isotype
IHC-P, F	H	180 to 240 kDa	Mouse	IgG1

Background: CD45 is a type I transmembrane protein with various extracellular domains due to alternative splicing and two conserved intracellular tyrosine phosphatase domains (1). The phosphatase activity of CD45 has been attributed to the first phosphatase domain while the second may interact/stabilize with the first domain or recruit/bind substrates (2-3). CD45 dephosphorylates Lck and Fyn at their conserved negative regulatory carboxy-terminal tyrosine residues, thus upregulating the activity of these Src-family kinases. Conversely, numerous studies indicate that CD45 inhibits Lck and Fyn by dephosphorylating their positive regulatory autophosphorylation site. CD45, therefore, appears to be both a positive and a negative regulator, conducting signals dependent on stimuli and cell type (1). Human leukocytes including lymphocytes, eosinophils, monocytes, basophils and neutrophils express CD45, in contrast to erythrocytes and platelets which are negative for CD45 expression (4).

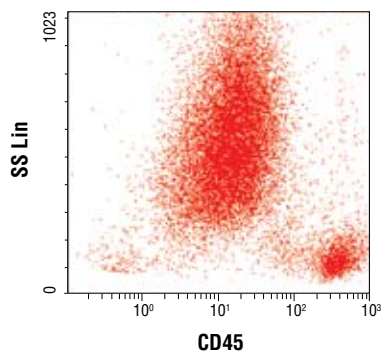


Immunohistochemical analysis of paraffin-embedded human tonsil, using CD45 (136-4B5) Mouse mAb.

Specificity/Sensitivity: CD45 (136-4B5) Mouse mAb detects endogenous levels of total CD45 protein.

Source/Purification: Monoclonal antibody is produced by immunizing BALB/c mice with stimulated human leukocytes.

Directions for Use: Incubate cells with 10% normal serum from same species as the secondary antibody for 10 minutes. Add the antibody at the recommended dilution and incubate for 30 minutes at room temperature. Wash with PBS and incubate with fluorochrome-conjugated secondary antibody for 30 minutes at room temperature. Wash with PBS and analyze on flow cytometer. Note: Not recommended for use with fixation/permeabilization protocols that contain methanol.



Flow cytometric analysis of whole blood, using CD45 (136-4B5) Mouse mAb.

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.

Storage: Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA, 50% glycerol and less than 0.02% sodium azide. Store at -20°C. Do not aliquot the antibody.

Recommended Antibody Dilutions:
 Immunohistochemistry (Paraffin) 1:50
IHC Protocol: Citrate/TBST
 Flow Cytometry 1:25

- Companion Products:**
- Phospho-Zap-70 (Tyr319)/Syk (Tyr352) Antibody #2701
 - Phospho-Zap-70 (Tyr493) Antibody #2704
 - Zap-70 (136F12) Rabbit mAb (Alexa Fluor® 647 Conjugate) #2707
 - Lck Antibody #2752
 - SLP-76 Antibody #4958
 - CD10 (CB-CALLA) Mouse mAb #3565
 - CD13 (B-F10) Mouse mAb #3566
 - CD16 (FcγIII) (CB-16) Mouse mAb #3567
 - CD34 (IC0115) Mouse mAb #3569
 - CD56 (NCAM) (123C3) Mouse mAb #3576

Immunohistochemistry Protocol (Paraffin)

***IMPORTANT:** See product data sheet for the appropriate wash buffer and antigen unmasking procedure.

- For Citrate/PBST, use steps 5a, 6a and C1.
- For Citrate/TBST, use steps 5b, 6a and C1.
- For EDTA/PBST, use steps 5a, 6b and C2.
- For EDTA/TBST, use steps 5b, 6b and C2.

A Solutions and Reagents

1. Xylene
2. Ethanol, anhydrous denatured, histological grade (100% and 95%)
3. Deionized water (dH₂O)
4. Hematoxylin (optional)
5. ***Wash Buffer:**
 - a. **For Citrate/PBST OR EDTA/PBST:** 1X PBS/0.1% Tween-20 (wash buffer): To prepare 1 L add 100 ml 10X PBS to 900 ml dH₂O. Add 1ml Tween-20 and mix.
 - 10X Phosphate Buffered Saline (PBS):** To prepare 1 L add 80 g sodium chloride (NaCl), 2 g potassium chloride (KCl), 14.4 g sodium phosphate, dibasic (Na₂HPO₄) and 2.4 g potassium phosphate, monobasic (KH₂PO₄) to 1 L dH₂O. Adjust pH to 7.4.
 - b. **For Citrate/TBST OR EDTA/TBST:** 1X TBS/0.1% Tween-20 (wash buffer): To prepare 1 L add 100 ml 10X TBS to 900 ml dH₂O. Add 1 ml Tween-20 and mix.
 - 10X Tris Buffered Saline (TBS):** To prepare 1 L add 24.2 g Trizma® base (C₄H₁₁NO₃) and 80 g sodium chloride (NaCl) to 1 L dH₂O. Adjust pH to 7.6 with concentrated HCl.
6. ***Antigen Unmasking Solution:**
 - a. **For Citrate/PBST OR Citrate/TBST:** 10 mM Sodium Citrate Buffer: To prepare 1 L, add 2.94 g sodium citrate trisodium salt dihydrate (C₆H₅Na₃O₇•2H₂O) to 1 L dH₂O. Adjust pH to 6.0.
 - b. **For EDTA/PBST OR EDTA/TBST:** 1 mM EDTA: To prepare 1 L add 0.372 g EDTA (C₁₀H₁₆N₂O₈Na₂•2H₂O) to 1 L dH₂O. Adjust pH to 8.0.
 - c. **Alternative Unmasking: 10 mM Tris:** To prepare 1 L add 1.21 g Trizma® Base (C₄H₁₁NO₃) to 1 L dH₂O. Adjust pH to 10.0.
7. **3% Hydrogen Peroxide:** To prepare, add 10 ml 30% H₂O₂ to 90 ml dH₂O.
8. **Blocking Solution:** 5% horse serum or goat serum diluted in recommended wash buffer.
9. Biotinylated secondary antibody.
10. **ABC Reagent:** (Vectastain ABC Kit, Vector Laboratories, Inc., Burlingame, CA) Prepare according to manufacturer's instructions 30 minutes before use.
11. **DAB Reagent or suitable substrate:** Prepare according to manufacturer's recommendations.

B Deparaffinization/Rehydration

NOTE: Do not allow slides to dry at any time during this procedure.

NOTE: Consult product data sheet for recommended wash buffer.

1. **Deparaffinize/hydrate sections:**
 - a. Incubate sections in three washes of xylene for 5 minutes each.
 - b. Incubate sections in two washes of 100% ethanol for 10 minutes each.
 - c. Incubate sections in two washes of 95% ethanol for 10 minutes each.
2. Wash sections twice in dH₂O for 5 minutes each.

C *Antigen Unmasking

NOTE: Consult product data sheet for specific recommendation for the unmasking solution.

1. **For Citrate/PBST OR Citrate/TBST:** Bring slides to a boil in 10 mM sodium citrate buffer pH 6.0 then maintain at a sub-boiling temperature for 10 minutes. Cool slides on bench top for 30 minutes.
2. **For EDTA/PBST OR EDTA/TBST:** Bring slides to a boil in 1 mM EDTA pH 8.0 followed by 15 minutes at a sub-boiling temperature. No cooling is necessary.
3. **Alternate:** Bring slides to a boil in 10 mM Tris pH 10.0 followed by 10 minutes at a sub boiling temperature. Cool slides on bench top for 30 minutes.

D Staining

1. Wash sections in dH₂O three times for 5 minutes each.
2. Incubate sections in 3% hydrogen peroxide for 10 minutes.
3. Wash sections in dH₂O twice for 5 minutes each.

NOTE: Consult product data sheet for recommended wash buffer.

4. Wash section in wash buffer for 5 minutes.
5. Block each section with 100–400 µl blocking solution for 1 hour at room temperature.
6. Remove blocking solution and add 100–400 µl diluted primary antibody to each section. (Dilute antibody in blocking solution.) Incubate overnight at 4°C.
7. Remove antibody solution and wash sections in wash buffer three times for 5 minutes each.
8. Add 100–400 µl secondary antibody, diluted in blocking solution per manufacturer's recommendation, to each section. Incubate 30 minutes at room temperature.
9. If using ABC avidin/biotin method, make ABC reagent according to the manufacturer's instructions and incubate solution for 30 minutes at room temperature.
10. Remove secondary antibody solution and wash sections three times with wash buffer for 5 minutes each.
11. Add 100–400 µl ABC reagent to each section and incubate for 30 minutes at room temperature.
12. Remove ABC reagent and wash sections three times in wash buffer for 5 minutes each.
13. Add 100–400 µl DAB or suitable substrate to each section and monitor staining closely.
14. As soon as the sections develop, immerse slides in dH₂O.
15. If desired, counterstain sections in hematoxylin per manufacturer's instructions.
16. Wash sections in dH₂O two times for 5 minutes each.
17. Dehydrate sections:
 - a. Incubate sections in 95% ethanol two times for 10 seconds each.
 - b. Repeat in 100% ethanol, incubating sections two times for 10 seconds each.
 - c. Repeat in xylene, incubating sections two times for 10 seconds each.
18. Mount coverslips.