

Certificate of Analysis

Anti-CD3 ϵ , clone APA1/1

(mouse monoclonal IgG₁)

Catalog # 05-785

Lot # 32513

Immunogen: Purified human CD3 ϵ . Accession # NP_000724.

Specificity: Recognizes CD3 ϵ , Mr 23kDa. Clone APA1/1 has been demonstrated to recognize a site on CD3 ϵ that is exposed upon engagement of the T-cell Receptor complex.² Exposure of this neopeptide precedes CD3 phosphorylation and recruitment and activation of ZAP-70³, which initiates the signaling cascade produced by T-cell activation. As such, clone APA1/1 provides the earliest known marker for T-cell receptor engagement, and thus T-cell activation.

Species Cross-reactivity: Human and mouse.

Formulation: 200 μ g of protein G purified mouse IgG₁ in 391 μ l of 70% storage buffer (0.1M Tris-glycine, pH 7.4, 0.15M NaCl, 0.05% sodium azide) and 30% glycerol. Liquid at -20°C.

Storage and Stability: Stable for 2 years at -20°C from date of shipment. For maximum recovery of product, centrifuge the vial prior to removing the cap.

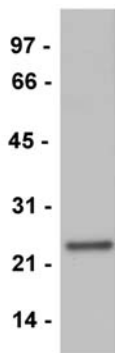
Handling Recommendations: Upon first thaw, and prior to removing the cap, centrifuge the vial and gently mix the solution. Aliquot into microcentrifuge tubes and store at -20°C. **Avoid repeated freeze/thaw cycles, which may damage IgG and affect product performance.** Note: Variability in freezer temperatures below -20°C may cause glycerol-containing solutions to become frozen during storage.

FOR RESEARCH USE ONLY - NOT FOR USE IN HUMANS

Quality Control Testing

Immunoblot Analysis: 0.5-2 μ g/ml of this lot detected CD3 ϵ in RIPA lysates from Jurkat cells.

Included Positive Antigen Control: Catalog # 12-303, Jurkat lysate. **Add 2.5 μ l of 2-mercaptoethanol/100 μ l of lysate and boil for 5 minutes to reduce the preparation.** Load 20 μ g of reduced lysate per lane for minigels.



Immunoblot Analysis

Representative blot from a previous lot. Jurkat cell lysate was resolved by electrophoresis, transferred to nitrocellulose and probed with anti-CD3 ϵ (0.5 μ g/ml). Proteins were visualized using a goat anti-mouse secondary antibody conjugated to HRP and a chemiluminescence detection system. Arrow indicates CD3 ϵ (~23kDa).

Additional Research Applications

Immunocytochemistry and Flow Cytometry: This antibody has been reported by an independent laboratory to stain CD3 ϵ in T cells whose T cell receptor has been engaged.

Immunoprecipitation: This antibody has been reported by an independent laboratory to precipitate CD3 ϵ from COS cells transfected with human CD3 ϵ .²

Application References:

1. Alarcon, B. *et al.*, *EMBO J.* **10**: 903-912, 1991.
2. Borroto, A. *et al.*, *J. Biol. Chem.* **273**: 12807-12816, 1998.
3. Gil, D. *et al.*, *Cell* **109**: 901-912, 2002.

General References:

4. Alarcon, B. *et al.*, *Immunol. Rev.* **191**: 38-46, 2003.
5. Werlen, G. *et al.*, *Science* **299**: 1859-1863, 2003.

Immunoblot Protocol

1. Perform SDS-polyacrylamide gel electrophoresis (SDS-PAGE) on cell lysate and transfer the proteins to nitrocellulose. Wash the blotted nitrocellulose twice with water.
2. Block the blotted nitrocellulose in freshly prepared PBS containing 3% nonfat dry milk (Catalog # 20-200), (PBS-MLK) for 1 hour at room temperature with constant agitation.
3. Incubate the nitrocellulose with **0.5-2 μ g/ml of anti-CD3 ϵ** , diluted in freshly prepared PBS-MLK overnight with agitation at 4°C.
4. Wash the nitrocellulose twice with water.
5. Incubate the nitrocellulose in the secondary reagent of choice (a goat anti-mouse HRP conjugated IgG, Catalog # 12-349, 1:5000 dilution was used) in PBS-MLK for 1 hour with agitation at room temperature.
6. Wash the nitrocellulose twice with water.
7. Wash the nitrocellulose in PBS-0.05% Tween[®]-20 for 3-5 minutes.
8. Rinse the nitrocellulose in 4-5 changes of water.
9. Use detection method of choice (enhanced chemiluminescence was used).