

Store at 4°C
and -20°C

#70976

Human Anti-CD3/CD28 T Cell Activation Kit

1 Kit
(10 assays)



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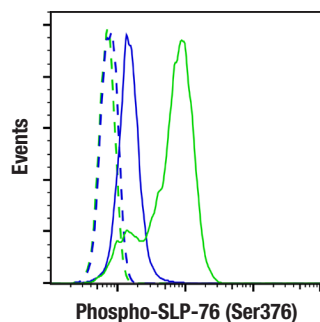
Components Ship As: 85363S	Item #	Kit Quantity	Storage Temp
Human CD3ε Activating (OKT3) Mouse mAb (Low Endotoxin, Azide-free)	92511	1 x 100 µg	4°C
Human CD28 Activating (CD28.2) Mouse mAb (Low Endotoxin, Azide-free)	91920	1 x 100 µg	4°C

Component Ships As: 75861S	Item #	Kit Quantity	Storage Temp
Goat Anti-Mouse Kappa Light Chain, F(ab') ₂ Antibody (Low Endotoxin, Azide-free)	75861	1 x 1 mL	-20°C

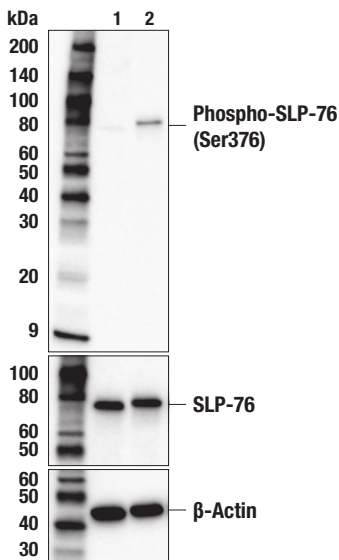
Description: The Human Anti-CD3/CD28 T Cell Activation Kit provides the necessary antibodies to induce human T cell activation and proliferation using the soluble stimulation protocol.

Specificity/Sensitivity: The Human Anti-CD3/CD28 T Cell Activation Kit is used for the stimulation of human T cells following the soluble stimulation protocol.

Background: T cells are activated by signaling through ITAM (immunoreceptor tyrosine-based activation motif)-containing CD3 signaling chains that associate with the T cell receptor (1). Co-stimulation through CD28 on T cells provides an additional signal required for effective T cell activation (2). Signaling through both CD3 and CD28 can be initiated using the agonist antibodies Human CD3ε Activating (OKT3) Mouse mAb (Low Endotoxin, Azide-free) and Human CD28 Activating (CD28.2) Mouse mAb (Low Endotoxin, Azide-free), respectively. If the agonist antibodies are used in the soluble stimulation protocol, cross-linking of their Fc domains using Goat Anti-Mouse Kappa Light Chain, F(ab')₂ Antibody (Low Endotoxin, Azide-free) is required for optimal stimulation.



Flow cytometric analysis of Jurkat cells, untreated (blue) or treated with cross-linked Human CD3ε Activating (OKT3) Mouse mAb (Low Endotoxin, Azide-free) #92511 and Human CD28 Activating (CD28.2) Mouse mAb (Low Endotoxin, Azide-free) #91920 (10 µg/mL each, 15 min; green), using Phospho-SLP-76 (Ser376) (E3G9U) XP[®] Rabbit mAb #76384 (solid lines) or concentration-matched Rabbit (DA1E) mAb IgG XP[®] Isotype Control #3900 (dashed lines). Anti-rabbit IgG (H+L), F(ab')₂ Fragment (Alexa Fluor[®] 488 Conjugate) #4412 was used as a secondary antibody.



Western blot analysis of extracts from Jurkat cells, untreated (lane 1) or treated with cross-linked Human CD3ε Activating (OKT3) Mouse mAb (Low Endotoxin, Azide-free) #92511 and Human CD28 Activating (CD28.2) Mouse mAb (Low Endotoxin, Azide-free) #91920 (10 µg/mL; 30 min) (lane 2), using Phospho-SLP-76 (Ser376) (D7S1K) Rabbit mAb #92711 (upper), SLP-76 (E4N7E) Rabbit mAb #25361 (middle), or β-Actin (D6A8) Rabbit mAb #8457 (lower).

Storage: All components in this kit are stable for 12 months when stored at the recommended temperature.

Background References:

- (1) Pitcher, L.A. and van Oers, N.S. (2003) *Trends Immunol* 24, 554-60.
- (2) June, C.H. et al. (1990) *Immunol Today* 11, 211-6.

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Human T Cell Stimulation Protocol

A. Solutions and Reagents

1. **Human CD3 ϵ Activating (OKT3) Mouse mAb (Low Endotoxin, Azide-free) #92511**
2. **Human CD28 Activating (CD28.2) Mouse mAb (Low Endotoxin, Azide-free) #91920**
3. **For Soluble Stimulation Only: Goat Anti-Mouse Kappa Light Chain, F(ab')₂ Antibody (Low Endotoxin, Azide-free) #75861**

B. Stimulation Protocol: Soluble

1. Collect cells of interest by centrifugation.
2. Aspirate the supernatant.
3. Resuspend cells at 10⁶-10⁷ cells/mL with cold serum-free media.
4. Add CD3 ϵ Activating (OKT3) Mouse mAb (Low Endotoxin, Azide-free) #92511 to cells at 1:200 to reach a final concentration of 10 μ g/mL.
5. Optional: Add Human CD28 Activating (CD28.2) Mouse mAb (Low Endotoxin, Azide-free) #91920 to cells at 1:200 to reach a final concentration of 10 μ g/mL.
6. Keep cells with agonist antibody on ice for 30 minutes.
7. Collect cells by centrifugation.
8. Aspirate the supernatant.
9. Resuspend cells at 10⁶-10⁷ cells/mL with cold serum-free media and keep on ice.
10. Add Goat Anti-Mouse Kappa Light Chain, F(ab')₂ Antibody (Low Endotoxin, Azide-free) #75861 secondary antibody to cells at 1:50 to reach a final concentration of 10 μ g/mL if only CD3 ϵ (OKT3) was used; add at 1:25 to reach a final concentration of 20 μ g/mL if both CD3 ϵ (OKT3) and CD28 (CD28.2) were used.
11. Keep cells with secondary antibody on ice for 15 minutes.
12. To initiate stimulation, transfer cells to 37°C water bath for desired stimulation time.
13. To terminate stimulation, follow the cell preparation protocol recommended for the desired analysis method.

C. Stimulation Protocol: Plate-based

1. Prepare a 10 μ g/mL solution of CD3 ϵ Activating (OKT3) Mouse mAb (Low Endotoxin, Azide-free) #92511 in sterile PBS. Prepare a sufficient volume to cover the bottom of all the wells to be used for the stimulated T cell condition (approximately 50 μ L for 96-well plates, 1 mL for 6-well plates).
2. Add the diluted CD3 ϵ (OKT3) antibody solution to the wells chosen for treatment. Add equivalent volume of sterile PBS to the wells chosen as controls for treatment.
3. Cover the plate and incubate at 37°C for a minimum of 2 hrs. The plate may be allowed to incubate overnight if desired.
4. Prepare cells as needed to generate a cell suspension of roughly 1 x 10⁶ cells/mL in a sufficient volume of complete media (100 μ L per well for 96-well plates, 2 mL for 6-well plates). Optimal cell density may be determined through experimentation with cells of interest. For T cell expansion, add Human Interleukin-2 (hIL-2) #8907 to the cell suspension to yield a final concentration of 20 ng/mL.
5. Aspirate the CD3 ϵ (OKT3) antibody solution from the wells, and rinse wells 2x with excess sterile PBS to remove unbound antibody.
6. Add cell suspension to all wells, at volumes specified in step 4 or as determined by experimentation.
7. Add Human CD28 Activating (CD28.2) Mouse mAb (Low Endotoxin, Azide-free) #91920 to yield a concentration of 2 μ g/mL. Pre-dilute CD28 (CD28.2) antibody in complete media if needed. CD28 antibody may also be added to a portion of control wells if desired.
8. Incubate cells for desired time in a humidified incubator (37°C, 5% CO₂). Add fresh media with IL-2 as needed for T cell expansion.
9. Harvest cells and proceed with immunostaining or analysis as desired.