

# **MOLECULAR PROBES®**

# PRODUCT INSERT

# HAMSTER anti-MOUSE CD28

Product Code	Form	Volume	Antibody*	Excitation (nm)	Peak Emission (nm)	Matching Isotype Controls	
HM3500	Purified	1.0 ml	200 µg	N/A	N/A	Hamster IgG Purified	Code HM00
HM3500-5	Purified	2.5 ml	500 μg				
HM3515	Biotin	1.0 ml	100 µg	N/A	N/A	Hamster IgG Biotin	Code HM15
HM3515-3	Biotin	3.0 ml	300 µg				
HM3501	FITC	1.0 ml	100 µg	488	525	Hamster IgG FITC	Code HM01
HM3501-3	FITC	3.0 ml	300 µg				
HM3504	R-PE	0.5 ml	50 µg	488	575	Hamster IgG R-PE	Code HM04
HM3504-3	R-PE	3.0 ml	300 µg				

#### PRODUCT DESCRIPTION

Hamster monoclonal antibody to mouse CD28

Clone: 37.51.1 Isotype: Hamster IgG

**Immunogen:** Mouse T lymphoma EL-4 cells<sup>1</sup>

Lot No.: See label Expiration: See label

**Buffer:** Phosphate buffered saline (PBS)

**Preservatives:** 0.1% *sodium azide*. Sodium azide is an extremely toxic and dangerous compound particularly when combined with acids or metals. Solutions containing sodium azide should be disposed of properly.

**Stabilizer:** For conjugated products only, a highly purified grade of BSA has been added as a stabilizing protein.

## **STORAGE & HANDLING**

Store reagents at 2-8°C. Light exposure should be avoided with fluorochrome conjugated reagents. Use dim light during handling, incubation with cells and prior to analysis. It is recommended that cells be analyzed within 18 hours of staining. If the reagent is being diluted, it is recommended that only the quantity to be used within one week be diluted.

## PRODUCT CHARACTERIZATION

**Antigen Specificity:** The 37.51.1 monoclonal antibody (mAb) reacts with CD28 which is weakly expressed by most T cells and NK cells<sup>1,2</sup>. Expression of CD28 by thymocytes and splenic T cells is increased after activation<sup>1</sup>. CD28 is a co-stimulatory receptor that interacts with CD80 (B7-1) and CD86 (B7-2) on antigen presenting cells<sup>3</sup>. The 37.51.1 mAb enhances cytokine production and proliferation of T and NK cells in the presence of certain mitogens<sup>1,2</sup>. Other applications of 37.51.1 mAb are immunostaining for flow cytometry and immunoprecipitation<sup>1</sup>.

#### PRODUCT QUALITY CONTROL

PI: L11257

Every lot is tested by flow cytometry using freshly harvested mouse lymph node cells. From this testing it is recommended that between 0.25 and 0.5  $\mu$ g of antibody be used per 1 x 10<sup>6</sup> cells in a 100  $\mu$ l staining volume. When staining with 37.51.1, it is suggested that Fc receptors be pre-blocked with purified mouse CD16/32 antibody (cat. # MFCR00) to

reduce Fc receptor-mediated non-specific binding. Because conditions may vary, it is recommended that each investigator determine the optimal amount of antibody to be used for each application.



Decreased background staining of 37.51.1 mAb using purified anti mouse CD16/32 (Cat. # MFCR00).

Briefly, one million cells from a single cell suspension of C57BL/6 splenocytes were simultaneously stained with 0.25µg of anti-mouse CD3 conjugated to APC (Cat.# RM3405) and 0.25µg of anti-mouse CD28 conjugated to FITC (Cat.# HM3501) either with preblocking of Fc receptors for 10 minutes using 0.5µg of purified anti-mouse CD16/32 (Fig.A) or without preblocking Fc receptors (Fig.B). Note the decreased staining of FITC conjugated anti-mouse CD28 on CD3 negative cells when Fc receptors are blocked.

#### **REFERENCES:**

- Gross, J. A., E. Callas, and J. P. Allison. 1992. Identification and distribution of the costimulatory receptor CD28 in the mouse. J. Immunol. 149: 380-388.
- Nandi, D., J. A. Gross, and J. P. Allison. 1994. CD28-mediated costimulation is necessary for optimal proliferation of murine NK cells. *J. Immunol.* 152: 3361-3369.
- 3. Bluestone, J. A. 1995. New perspectives of CD28-B7-mediated T cell costimulation. *Immunity* 2: 555-559.
- \* The amount of antibody is determined by measuring the optical density using a spectrophotometer. The antibody titer is verified by immunofluorescent staining and flow cytometric analysis.

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Invitrogen Corporation • 542 Flynn Rd • Camarillo • CA 93012 • Tel: 800.955.6288 • E-mail: techsupport@invitrogen.com

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