

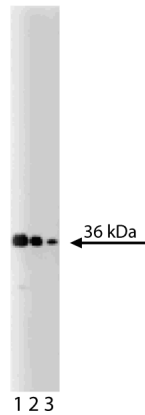
Technical Data Sheet

Purified Mouse Anti-Psme3/PA28-γ**Product Information**

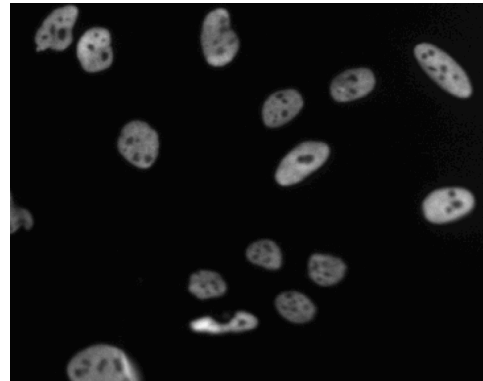
Material Number:	611180
Alternate Name:	PA28-γ
Size:	50 µg
Concentration:	250 µg/ml
Clone:	47/Psme3
Immunogen:	Mouse Psme3/PA28-γ aa. 45-147
Isotype:	Mouse IgG1
Reactivity:	QC Testing: Rat Tested in Development: Dog, Human, Mouse
Target MW:	36 kDa
Storage Buffer:	Aqueous buffered solution containing BSA, glycerol, and ≤0.09% sodium azide.

Description

The 20S proteasome is the major constituent of the proteasomal complex that mediates degradation events, including the generation of antigenic peptides that interact with MHC class I. The 20S proteasome is a cylindrical shaped complex composed of four layers of rings, each with seven subunits. The inner rings contain α-subunits, while the outer rings contain β-catalytic subunits. The constituents of the PA28 activator complex are additional subunits with specialized roles in class I-mediated antigen presentation. PA28 is a ring-shaped structure with alternating α- and β-subunits. This complex binds the α-rings of 20S and stimulates its activity. Expression of the PA28 α- and β-subunits is strongly induced by IFN-γ. The protein product of the Psme3 gene, also known as the Ki antigen, is highly homologous to the PA28 α- and β-subunits and has been designated the PA28 γ-subunit. This protein forms a homohexamer that binds the 20S proteasome and is thought to modulate proteasome activity. The PA28 α- and β-subunits are located in the cytoplasm and in the nucleus, while the γ-subunit is almost exclusively nuclear.



Western blot analysis of Psme3 on a rat cerebrum lysate. Lane 1: 1:1000, lane 2: 1:2000, lane 3: 1:4000 dilution of the Psme3 antibody.



Immunofluorescent staining of HeLa (ATCC CCL-2) cells. Cells were seeded in a 96 well imaging plate (Cat. No. 353219) at ~ 10 000 cells per well. After overnight incubation, cells were stained using the alcohol perm protocol and the anti-Psme3/PA28-γ antibody. The second step reagent was FITC goat anti mouse Ig (Cat. No. 554001). The image was taken on a BD Pathway™ 855 Bioimager using a 20x objective. This antibody also stained A549 (ATCC CCL-185) and U-2 OS (ATCC HTB-96) cells using both the Triton™ X-100 and alcohol perm protocols (see Recommended Assay Procedure).

Preparation and Storage

Store undiluted at -20°C.

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.

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Application Notes

Application

Western blot	Routinely Tested
Bioimaging	Tested During Development

Recommended Assay Procedure:

Bioimaging

1. Seed the cells in appropriate culture medium at ~10,000 cells per well in a BD Falcon™ 96-well Imaging Plate (Cat. No. 353219) and culture overnight.
2. Remove the culture medium from the wells, and fix the cells by adding 100 µl of BD Cytofix™ Fixation Buffer (Cat. No. 554655) to each well. Incubate for 10 minutes at room temperature (RT).
3. Remove the fixative from the wells, and permeabilize the cells using either BD Perm Buffer III, 90% methanol, or Triton™ X-100:
 - a. Add 100 µl of -20°C 90% methanol or Perm Buffer III (Cat. No. 558050) to each well and incubate for 5 minutes at RT.OR
 - b. Add 100 µl of 0.1% Triton™ X-100 to each well and incubate for 5 minutes at RT.
4. Remove the permeabilization buffer, and wash the wells twice with 100 µl of 1× PBS.
5. Remove the PBS, and block the cells by adding 100 µl of BD Pharmingen™ Stain Buffer (FBS) (Cat. No. 554656) to each well. Incubate for 30 minutes at RT.
6. Remove the blocking buffer and add 50 µl of the optimally titrated primary antibody (diluted in Stain Buffer) to each well, and incubate for 1 hour at RT.
7. Remove the primary antibody, and wash the wells three times with 100 µl of 1× PBS.
8. Remove the PBS, and add the second step reagent at its optimally titrated concentration in 50 µl to each well, and incubate in the dark for 1 hour at RT.
9. Remove the second step reagent, and wash the wells three times with 100 µl of 1× PBS.
10. Remove the PBS, and counter-stain the nuclei by adding 200 µl per well of 2 µg/ml Hoechst 33342 (e.g., Sigma-Aldrich Cat. No. B2261) in 1× PBS to each well at least 15 minutes before imaging.
11. View and analyze the cells on an appropriate imaging instrument.

Bioimaging: For more detailed information please refer to http://www.bdbiosciences.com/support/resources/protocols/ceritified_reagents.jsp

Western blot: For more detailed information please refer to http://www.bdbiosciences.com/pharmingen/protocols/Western_Blotting.shtml

Suggested Companion Products

Catalog Number	Name	Size	Clone
554001	FITC Goat Anti-Mouse Ig	0.5 mg	Polyclonal
554002	HRP Goat Anti-Mouse Ig	1.0 ml	(none)
611463	Rat Cerebrum Lysate	500 µg	(none)
353219	BD Falcon™ 96-well Imaging Plate	NA	(none)
554655	Fixation Buffer	100 ml	(none)
558050	Perm Buffer III	125 ml	(none)

Product Notices

1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
2. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.
3. This antibody has been developed and certified for the bioimaging application. However, a routine bioimaging test is not performed on every lot. Researchers are encouraged to titrate the reagent for optimal performance.
4. Caution: Sodium azide yields highly toxic hydrazoic acid under acidic conditions. Dilute azide compounds in running water before discarding to avoid accumulation of potentially explosive deposits in plumbing.
5. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
6. Triton is a trademark of the Dow Chemical Company.

References

Kohda K, Ishibashi T, Shimbara N, Tanaka K, Matsuda Y, Kasahara M. Characterization of the mouse PA28 activator complex gene family: complete organizations of the three member genes and a physical map of the approximately 150-kb region containing the alpha- and beta-subunit genes. *J Immunol.* 1998; 160(10):4923-4935. (Biology)