

## **Product datasheet**

# Alexa Fluor® 647 Anti-Melanoma gp100 antibody [EP4863(2)] ab246730

Recombinant RabMAb

2 References 2 Images

Overview Product name Alexa Fluor® 647 Anti-Melanoma gp100 antibody [EP4863(2)] Description Alexa Fluor® 647 Rabbit monoclonal [EP4863(2)] to Melanoma gp100 Host species Rabbit Alexa Fluor® 647. Ex: 652nm, Em: 668nm Conjugation **Tested applications** Suitable for: Flow Cyt (Intra), ICC/IF **Species reactivity** Reacts with: Mouse Immunogen Synthetic peptide. This information is proprietary to Abcam and/or its suppliers. **Positive control** ICC/IF: B16F10 cells. Flow Cyt (intra): B16F10 cells. **General notes** This product is a recombinant monoclonal antibody, which offers several advantages including: - High batch-to-batch consistency and reproducibility - Improved sensitivity and specificity - Long-term security of supply - Animal-free production For more information see here. Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb<sup>®</sup> patents**. Alexa Fluor<sup>®</sup> is a registered trademark of Molecular Probes, Inc, a Thermo Fisher Scientific Company. The Alexa Fluor<sup>®</sup> dye included in this product is provided under an intellectual property license from Life Technologies Corporation. As this product contains the Alexa Fluor® dye, the purchase of this product conveys to the buyer the non-transferable right to use the purchased product and components of the product only in research conducted by the buyer (whether the buyer is an academic or for-profit entity). As this product contains the Alexa Fluor<sup>®</sup> dye the sale of this product is expressly conditioned on the buyer not using the product or its components, or any materials made using the product or its components, in any activity to generate revenue, which may include, but is not limited to use of the product or its components: in manufacturing; (ii) to provide a service, information, or data in return for payment (iii) for therapeutic, diagnostic or prophylactic purposes; or (iv) for resale, regardless of whether they are sold for use in research.

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For information on purchasing a license to this product for purposes other than research, contact

The Life Science industry has been in the grips of a reproducibility crisis for a number of years. Abcam is leading the way in addressing this with our range of recombinant monoclonal antibodies and knockout edited cell lines for gold-standard validation. Please check that this product meets your needs before purchasing.

If you have any questions, special requirements or concerns, please send us an inquiry and/or contact our Support team ahead of purchase. Recommended alternatives for this product can be found below, along with publications, customer reviews and Q&As

#### **Properties**

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C. Avoid freeze / thaw cycle. Stable for 12 months at -20°C. Store In the Dark.
Storage buffer	pH: 7.40 Preservative: 0.02% Sodium azide Constituents: 30% Glycerol (glycerin, glycerine), 1% BSA, PBS
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EP4863(2)
lsotype	lgG

#### **Applications**

The Abpromise guarantee Our <u>Abpromise guarantee</u> covers the use of ab246730 in the following tested applications.

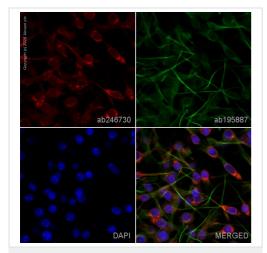
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
Flow Cyt (Intra)		1/5000.
ICC/IF		1/100. This product gave a positive signal in B16F10 fixed with 100% methanol (5 mins).

Target	
Function	Plays a central role in the biogenesis of melanosomes. Involved in the maturation of melanosomes from stage I to II. The transition from stage I melanosomes to stage II melanosomes involves an elongation of the vesicle, and the appearance within of distinct fibrillar structures. Release of the soluble form, ME20-S, could protect tumor cells from antibody mediated immunity.
Tissue specificity	Preferentially expressed in melanomas. Some expression was found in dysplastic nevi. Not found in normal tissues nor in carcinomas. Normally expressed at low levels in quiescent adult melanocytes but overexpressed by proliferating neonatal melanocytes and during tumor growth.
Sequence similarities	Belongs to the PMEL/NMB family. Contains 1 PKD domain.

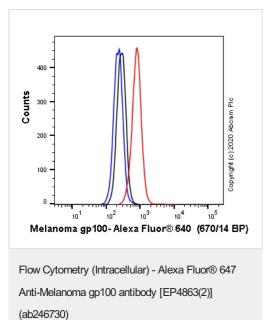
The RPT domain is essential for the generation of the fibrillar matrix of melanosomes. The lumenal domain is necessary for correct processing and trafficking to melanosomes.
A small amount of P1/P100 (major form) undergoes glycosylation to yield P2/P120 (minor form). P2 is cleaved by a furin-like proprotein convertase (PC) in a pH-dependent manner in a post- Golgi, prelysosomal compartment into two disulfide-linked subunits: a large lumenal subunit, M- alpha/ME20-S, and an integral membrane subunit, M-beta. Despite cleavage, only a small fraction of M-alpha is secreted, whereas most M-alpha and M-beta remain associated with each other intracellularly. M-alpha is further processed to M-alpha N and M-alpha C. M-alpha C further undergoes processing to yield M-alpha C1 and M-alpha C3 (M-alpha C2 in the case of PMEL17- is or PMEL17-Is). Formation of intralumenal fibrils in the melanosomes requires the formation of M-alpha that becomes incorporated into the fibrils. Stage II melanosomes harbor only Golgi- modified Pmel17 fragments that are derived from M-alpha and that bear sialylated O-linked oligosaccharides. N-glycosylated. O-glycosylated; contains sialic acid.
Secreted and Endoplasmic reticulum membrane. Golgi apparatus. Melanosome. Endosome > multivesicular body. Identified by mass spectrometry in melanosome fractions from stage I to stage IV. Localizes predominantly to intralumenal vesicles (ILVs) within multivesicular bodies. Associates with ILVs found within the lumen of premelanosomes and melanosomes and particularly in compartments that serve as precursors to the striated stage II premelanosomes.

### Images



Immunocytochemistry/ Immunofluorescence - Alexa Fluor® 647 Anti-Melanoma gp100 antibody [EP4863(2)] (ab246730) ab246730 staining Melanoma gp100 in B16F10 cells. The cells were fixed with 100% methanol (5 min), permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated overnight at +4°C with ab246730 at 1/100 dilution (shown in red) and **ab195887**, Mouse monoclonal to alpha Tubulin (Alexa Fluor<sup>®</sup> 488), at 1/250 dilution (shown in ). Nuclear DNA was labelled with DAPI (shown in blue).

Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).



Overlay histogram showing B16F10 cells stained with ab246730 (red line). The cells were fixed with 80 % methanol (5 min) and then permeabilized with 0.1 % PBS-Triton X-100 for 15 min. The cells were then incubated in 1x PBS containing 10 % normal goat serum to block non-specific protein-protein interaction followed by the antibody (ab246730) (1x  $10^6$  in  $100\mu$ I at  $0.1\mu$ g/mI (1/5000)) for 30 min at  $22^{\circ}$ C.

Isotype control antibody (black line) was Rabbit IgG (monoclonal) Alexa Fluor<sup>®</sup>647 (<u>ab199093</u>) used at the same concentration and conditions as the primary antibody. Unlabeled sample (blue line) was also used as a control.

Acquisition of >5,000 events were collected using a 40 mW Red laser (640nm) and 670/14 bandpass filter.

Please note: All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"

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