Datasheet for ABIN967667

anti-GATA4 antibody

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Overview

Quantity:	0.1 mg
Target:	GATA4
Reactivity:	Human, Mouse
Host:	Mouse
Clonality:	Monoclonal
Application:	Western Blotting (WB), BioImaging (BI)

Product Details

Brand:	BD Pharmingen™
Immunogen:	Human GATA4 Peptide
Clone:	L97-56
Isotype:	IgG1 kappa
Cross-Reactivity:	Mouse (Murine)
Characteristics:	1. Since applications vary, each investigator should titrate the reagent to obtain optimal results. 2. Please refer to us 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.

Product Details

	5. Triton is a trademark of the Dow Chemical Company.
Purification:	The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.

Target Details

Target:	GATA4
Alternative Name:	GATA4 (GATA4 Products)
Background:	The L97-56 monoclonal antibody reacts with GATA4 (GATA-binding protein 4), a member of the GATA family of zinc finger-containing transcription factors that bind to the GATA nucleotide sequence. This ~50-kDa (observed MW) nuclear protein is expressed in mesodermal and definitive endodermal tissues such as the gastrointestinal tract, gonads, and heart. Genetic studies suggest that GATA4 regulates embryonic cardiac development: in mice, disruption of the GATA4 gene leads to defects in heart tube formation, while mutations of GATA4 are associated with atrial septal defects in humans. In the adult heart, GATA4 regulates differentiated gene expression. The roles of GATA4 in endocrine and reproductive functions were recently reviewed. Synonyms: MGC126629, GATA-4
Molecular Weight:	42 - 50 kDa
Pathways:	Peptide Hormone Metabolism, Carbohydrate Homeostasis

Application Details

Application Notes:	Recommended Protocol for Bioimaging: 1. Seed the cells in appropriate culture medium at an appropriate cell density in an 96-well Imaging Plate , and culture overnight to 48 hours. 2. Remove the culture medium from the wells, and wash (one to two times) with 100 myl of 1× PBS. 3. Fix the cells by adding 100 µl of fresh 3.7% Formaldehyde in PBS or fixation buffer to each well and incubating for 10 minutes at room temperature (RT). 4. Remove the fixative from the wells, and wash the wells (one to two times) with 100 myl of 1× PBS. 5. Permeabilize the cells using either cold methanol (a), Triton™ X-100 (b), or Saponin (c): a. Add 100 µl of -20°C 90% methanol to each well and incubate for 5 minutes at RT. b. Add 100 µl of 0.1% Triton™ X-100 to each well and incubate for 5 minutes at RT. c. Add 100 µl of 1×

Perm/Wash buffer to each well and incubate for 15 to 30 minutes at RT. Continue to use 1× Perm/Wash buffer for all subsequent wash and dilutions steps.

6. Remove the permeabilization buffer from the wells, and wash one to two times with 100 myl of appropriate buffer (either 1× PBS or 1× Perm/Wash buffer, see step 5.c.).

7. Optional blocking step: Remove the wash buffers, and block the cells by adding 100 µl of blocking buffer or 3% FBS in appropriate dilution buffer to each well and incubating for 15 to 30 minutes at RT.

8. Dilute the antibody to its optimal working concentration in appropriate dilution buffer. Titrate purified (unconjugated) antibodies and second-step reagents to determine the optimal concentration. If using a Bioimaging Certified antibody conjugate, dilute it 1:10.

9. Add 50 µl of diluted antibody per well and incubate for 60 minutes at RT. Incubate in the dark if using fluorescently labeled antibodies.

10. Remove the antibody, and wash the wells three times with 100 myl of wash buffer. An optional detergent wash (100 myl of 0.05% Tween in 1× PBS) can be included prior to the regular wash steps.

11. If the antibody being used is fluorescently labeled, then move to step 12. Otherwise, if using a purified unlabeled antibody, repeat steps 8 to 10 with a fluorescently labeled second-step reagent to detect the purified antibody.

12. After the final wash, counter-stain the nuclei by adding 100 ml of a 2 mg/ml solution of Hoechst 33342 in 1× PBS to each well at least 15 minutes before imaging.

13. View and analyze the cells on an appropriate imaging instrument.

Recommended Assay Procedure for Tissue Sections:

1. Harvest target organs or tissues and flush with PBS.

2. Place the tissues in cassettes and fix with 10% neutral buffered formalin for 16 hrs.

3. Remove the fixative and proceed with processing and embedding in paraffin using standard protocols.

4. Cut paraffin-embedded tissue sections (5 µm) using a microtome, adhere sections onto colorfrost slides, and allow them to air dry.

5. Deparaffinize and re-hydrate the sections.

6. Treat with Retrievagen A by heating the slides in a pressure cooker at 121°C for 15 minutes at 17 psi.

7. Allow the slides to cool to room temperature in the Retrievagen A, rinse the slides with tap water, and store in PBS prior to antibody staining.

8. Sections can be simultaneously stained with a cocktail of multiple antibodies at pre-optimized concentration, for 2 hours at room temperature.

Application Details

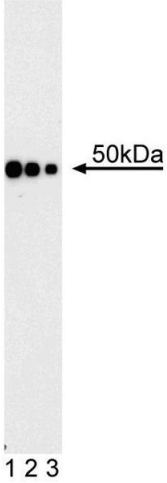
	9. Wash the sections in 1× PBS. 10. If required label cellular DNA with 2 µg/ml Hoechst 33342 for 30 minutes, and wash with 1× PBS. 11. Place cover slips on the sections using Aqua-Mount. 12. View and analyze the samples on an appropriate imaging instrument.
Restrictions:	For Research Use only

Handling

Format:	Liquid
Concentration:	0.5 mg/mL
Buffer:	Aqueous buffered solution containing ≤0.09 % sodium azide.
Preservative:	Sodium azide
Precaution of Use:	This product contains Sodium azide: a POISONOUS AND HAZARDOUS SUBSTANCE which should be handled by trained staff only.
Storage:	4 °C
Storage Comment:	Store undiluted at 4°C.

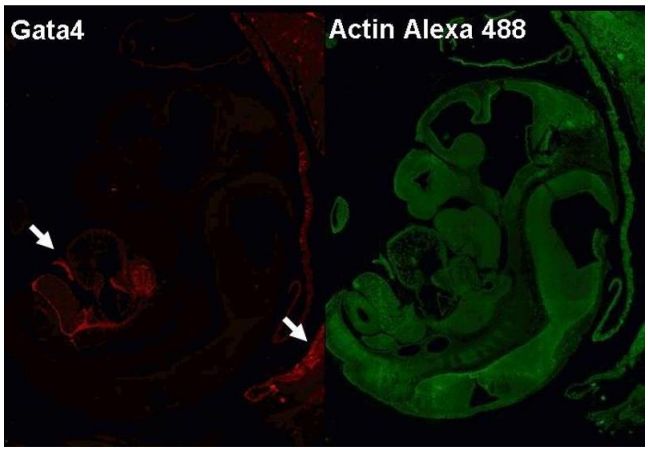
Publications

Product cited in:	Viger, Guittot, Anttonen, Wilson, Heikinheimo: "Role of the GATA family of transcription factors in endocrine development, function, and disease." in: **Molecular endocrinology (Baltimore, Md.)**, Vol. 22, Issue 4, pp. 781-98, (2008) (PubMed). Oka, Xu, Molkentin: "Re-employment of developmental transcription factors in adult heart disease." in: **Seminars in cell & developmental biology**, Vol. 18, Issue 1, pp. 117-31, (2007) (PubMed). Jonak, Baserga: "Cytoplasmic regulation of two G1-specific temperature-sensitive functions." in: **Cell**, Vol. 18, Issue 1, pp. 117-23, (1980) (PubMed).



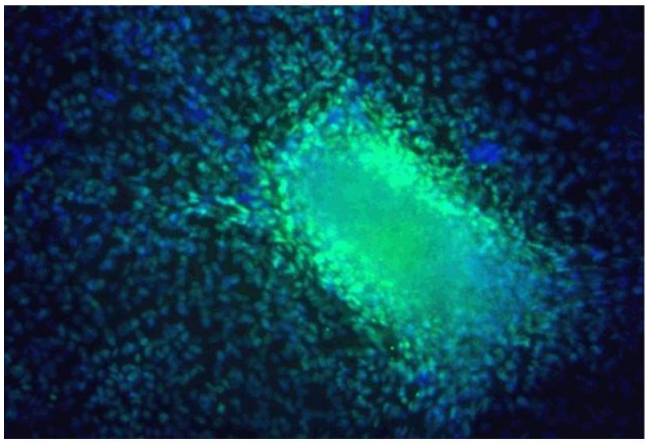
Western Blotting

Image 1. Western blot analysis using anti-human Gata4 antibody. Cell lysates were prepared from mouse embryonal carcinoma F9 (ATCC CRL-1720) cells treated with dibutryl cyclic AMP and retinoic acid. Western Blot was probed using the GATA4 monoclonal antibody at concentrations of 0.02, 0.01, and 0.005 µg/ml (lanes 1, 2, and 3, respectively). GATA4 is identified as a band of 50 kDa.



Immunohistochemistry (Paraffin-embedded Sections)

Image 2. Following antigen retrieval with Retrievagen A (pH 6.0), the formalin-fixed paraffin-embedded mouse E12 embryo section was stained with anti-GATA4 monoclonal antibody followed by Alexa Fluor® 555 goat anti-mouse Ig (Invitrogen) (pseudo-colored red), and Alexa Fluor® 488 Mouse anti-Actin (pseudo colored green) according to the Recommended Assay Procedure for tissue sections. The image was captured as a 6x6 montage with a 10x objective. A collapsed Z-stack is shown: arrows indicate the developing heart and extra embryonic endoderm.



Immunofluorescence

Image 3. Immunofluorescent staining of hESC H9 derived cardiomyocytes (First Panel) and mouse E12 embryo section (Second Panels). Left: Cells of the H9 cell line (WiCell, Madison, WI) were differentiated into cardiac mesoderm and plated on Permanox™ slides. The cells were fixed, permeabilized with BD Perm/Wash™ then stained with Purified Mouse anti-GATA4 (2µg/mL) according to the Recommended Assay Procedure. The second step reagent was Alexa Fluor® 555 goat anti-mouse Ig (Invitrogen) (pseudo colored green). Cell nuclei were counterstained with Hoechst 33342 (pseudo colored blue). The image was captured on a BD Pathway™ 435 High-Content Bioimager

System using a 20X objective, and merged using BD AttoVision™ software.

