Technical Data Sheet

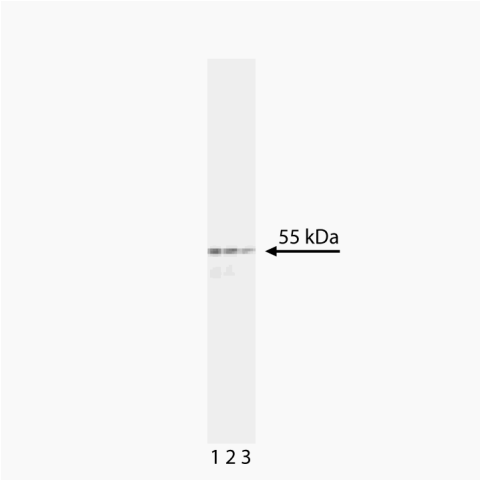
Purified Mouse Anti-Cdk8

Product Information

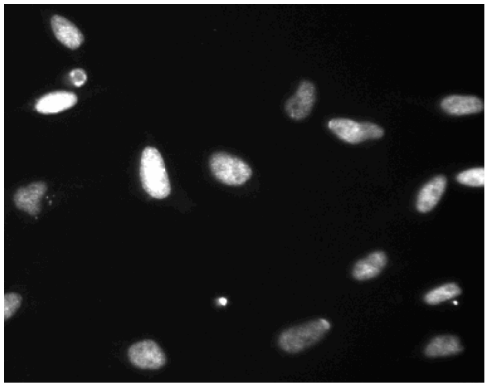
Material Number:	**552056**
Size:	50 µg
Reactivity:	QC Testing: Dog Tested in Development: Human, Mouse, Chicken
Target MW:	55 kD
Component:	**51-8130HR**
Description:	Purified Mouse Anti-Cdk8
Size:	50 µg (1 ea)
Concentration:	0.5 mg/ml
Clone Name:	F9-1075
Immunogen:	Human Cdk8 (C-terminal) Peptide
Isotype:	Mouse IgG1
Storage Buffer:	Aqueous buffered solution containing ≤0.09% sodium azide.

Description

Cyclins, cyclin-dependent kinases (Cdks), and cyclin-dependent kinase inhibitors (CdkIs) are essential for cell-cycle control in eukaryotes. Cyclins, regulatory subunits, bind to cyclin-dependent kinases (Cdks), catalytic subunits, to form active cyclin-Cdk complexes. Cdk subunits by themselves are inactive and binding to a cyclin is required for their activity. Cyclins A, B1, D and E undergo periodic synthesis and degradation, thereby providing a mechanism to regulate Cdk activity throughout the cell cycle. In contrast to other members of the cyclin family, cyclin C does not have a role in regulating cell cycle progression. Cyclin C and its partner, Cdk8 are associated with RNA polymerase II, an enzyme responsible for transcribing protein encoding genes. Research has shown that Cdk8 stabilizes cyclin C to prevent its degradation, moreover this stabilization exists for either the catalytically active or inactive form of Cdk8. This is in agreement with previous work showing that the kinase activity of the cyclin C - Cdk8 does not change during the cell cycle.



Western blot analysis of Cdk8 on a MDCK lysate (Canine Kidney; ATCC CCL-34). *Lane 1: 0.5 µg/mL, lane 2: 0.25 µg/mL, and lane 3: 0.125 µg/mL of the Mouse Anti-Cdk8 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-Cdk8 antibody. The second step reagent was Alexa Fluor® 555 goat anti-mouse IgG (Invitrogen). The image was taken on a BD Pathway™ 855 Bioimager system using a 20x objective. This antibody also stained A549 (ATCC CCL-185) and U-2 OS (ATCC HRB-96) cells and worked with both the Triton™ X-100 and alcohol perm protocols (see Recommended Assay Procedure).*

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Preparation and Storage

Store undiluted at 4°C.

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

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.

Suggested Companion Products

Catalog Number	Name	Size	Clone
611635	MDCK Cell Lysate	500 µg	(none)
554002	HRP Goat Anti-Mouse Ig	1 mL	(none)
353219	BD Falcon™ 96-well Imaging Plate		(none)
554655	Fixation Buffer	100 mL	(none)
558050	Perm Buffer III	125 mL	(none)
554656	Stain Buffer (FBS)	500 mL	(none)

Product Notices

1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
2. 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.
3. Triton is a trademark of the Dow Chemical Company.
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. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.

References

Barette C, Jariel-Encontre I, Piechaczyk M, Piette J. Human cyclin C protein is stabilized by its associated kinase cdk8, independently of its catalytic activity. *Oncogene.* 2001; 20(5):551-562. (Biology)

Johnson DG, Walker CL. Cyclins and cell cycle checkpoints. *Annu Rev Pharmacol Toxicol.* 1999; 39:295-312. (Biology)

Rickert P, Corden JL, Lees E. Cyclin C/CDK8 and cyclin H/CDK7/p36 are biochemically distinct CTD kinases. *Oncogene.* 1999; 18(4):1093-1102. (Biology)

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