Technical Data Sheet

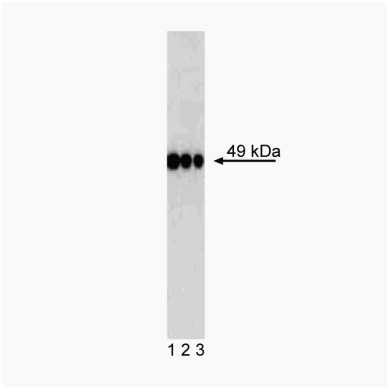
Purified Mouse anti-TAZ

Product Information

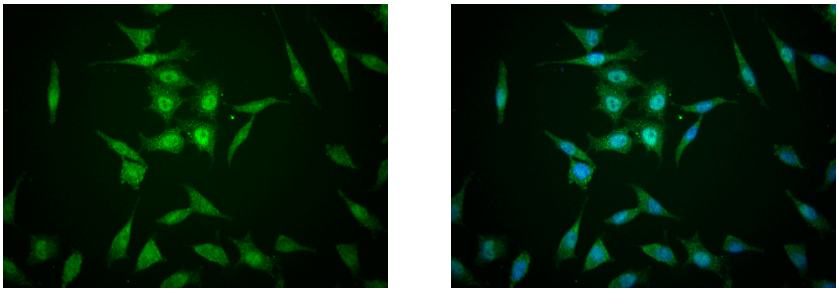
Material Number:	**560235**
Alternate Name:	WWTR1
Size:	0.1 mg
Concentration:	0.5 mg/ml
Clone:	M2-616
Immunogen:	Human TAZ Recombinant Protein
Isotype:	Mouse (BALB/c) IgG2b, κ
Reactivity:	QC Testing: Human Cross-reactivity: Mouse
Target MW:	49 kDa
Storage Buffer:	Aqueous buffered solution containing ≤0.09% sodium azide.

Description

Taz is a 49-kDa transcriptional co-activator with a PDZ-binding motif and is regulated by binding with 14-3-3 proteins. It plays a key role in differentiation of mesenchymal stem cells into either osteoblasts or adipocytes via interactions with key transcription factors Runx2 and PPARγ. More recently, Taz was found to be a component of an E3 ubiquitin ligase involved in ubiquitin-dependent substrate proteolysis by mediating its interaction with the F-box protein β-Trcp. Therefore, Taz has dual functions of regulating protein degradation and transcription.



Western blot analysis of TAZ in transformed human epithelioid carcinoma. *HeLa cell lysate (Cat. No.611449) was probed with Purified Mouse anti-TAZ monoclonal antibody at concentrations of 0.5, 0.25, and 0.125 µg/ml (lanes 1, 2, and 3, respectively). TAZ is identified as a band of 49 kDa.*



Immunofluorescent staining of human cell lines. *HeLa cells (ATCC CCL-2) were seeded in two 96-well imaging plates (Cat. No. 353219) at ~10,000 cells per well. After overnight incubation, the cells were fixed, permeabilized with Saponin, and stained with Purified Mouse anti-TAZ (pseudocolored green) according to the Recommended Assay Procedure. The second-step reagent was Alexa Fluor® 555 goat anti-mouse Ig (Invitrogen). The left image shows TAZ alone, and the right image shows TAZ merged with Hoechst staining. Images were captured on a BD Pathway™ 435 bioimager using a 20x objective and merged using BD AttoVision™ software. Other permeabilization methods, cold methanol and Triton™ X-100, did not work well.*

Preparation and Storage

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.
Store undiluted at 4°C.

Application Notes

Application

Western blot	Routinely Tested
Bioimaging	Tested During Development

Recommended Assay Procedure:

For more information, please refer to: http://www.bdbiosciences.com/pharmingen/protocols/Western_Blotting.shtml
and Bioimaging: http://www.bdbiosciences.com/pharmingen/protocols/Bioimaging_Certified.shtml

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Recommended Protocol for Bioimaging:

1. Seed the cells in appropriate culture medium at an appropriate cell density in a BD Falcon™ 96-well Imaging Plate (Cat. No. 353219), and culture overnight to 48 hours.
2. Remove the culture medium from the wells, and wash (one to two times) with 100 µl of 1× PBS.
3. Fix the cells by adding 100 µl of fresh 3.7% Formaldehyde in PBS or BD Cytofix™ fixation buffer (Cat. No. 554655) 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 µl 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 or -20°C BD™ Phosflow Perm Buffer III (Cat. No. 558050) 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 (Cat. No. 554723) 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 µl 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 BD Pharmingen™ Stain Buffer (FBS) (Cat. No. 554656) 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 µl of wash buffer. An optional detergent wash (100 µl 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 µl of a 2 µg/ml solution of Hoechst 33342 (eg, Sigma-Aldrich Cat. No. B2261) in 1× PBS to each well at least 15 minutes before imaging.
13. View and analyze the cells on an appropriate imaging instrument.

Suggested Companion Products

Catalog Number	Name	Size	Clone
554655	Fixation Buffer	100 ml	(none)
554656	Stain Buffer (FBS)	500 ml	(none)
353219	BD Falcon™ 96-well Imaging Plate	NA	(none)
554723	Perm/Wash Buffer	100 ml	(none)
554002	HRP Goat Anti-Mouse Ig	1.0 ml	(none)
611449	HeLa Cell Lysate	500 µg	(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. 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.
4. Triton is a trademark of the Dow Chemical Company.
5. 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.

References

Hong JH, Hwang ES, McManus MT, et al. TAZ, a transcriptional modulator of mesenchymal stem cell differentiation. *Science.* 2005; 309:1074-1078. (Biology)
Hong JH, Yaffe MB. TAZ: a beta-catenin-like molecule that regulates mesenchymal stem cell differentiation. *Cell Cycle.* 2006; 5(2):176-179. (Biology)
Kanai F, Marignani PA, Sarbassova D, et al. TAZ: a novel transcriptional co-activator regulated by interactions with 14-3-3 and PDZ domain proteins. *EMBO J.* 2000; 19(24):6778-6791. (Biology)

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