V 2.1

Anti-Tubulin Polyclonal Ab (sheep host)

Cat. # ATN02
Lot# 105
Upon arrival, store at 4°C (desiccated)
See datasheet for storage after reconstitution

Form:	Lyophilized powder
Amount of material:	1 x 25 µl when reconstituted,
Validated applications:	WB, IF
Species reactivity:	Wide range, including mammals, amphibi-
Host/Isotype:	Sheep/IgG
Clone:	Polyclonal

Background Information
Tubulin is composed of a heterodimer of two closely related 55 kDa proteins called alpha and beta tubulin (1). The two proteins are encoded by separate genes, or small gene families, whose sequences are highly conserved throughout the eukaryotic kingdom. This fact, plus the antigen design (see below) has allowed the generation of a polyclonal antibody that can detect tubulin originating from many diverse species (see Figure 1 and refs. 2-4).

Material
The anti-tubulin antibody (Cat. # ATN02) is an affinity-purified sheep polyclonal antibody that reacts to alpha and beta tubulin. The immunogen used for antibody production was a mixture of purified tubulins from a variety of species. Antibody ATN02 has broad species cross reactivity from yeast to humans. Brain extract (Part # EXT03) is included as a positive control. ATN02 identifies a characteristic tubulin band at 55 kDa on Western blots (see Fig. 1). ATN02 is supplied as a lyophilized white powder.

Storage and Reconstitution
Shipped at ambient temperature. The lyophilized protein can be stored desiccated at 4°C for 6 months. For reconstitution, the product tube should be briefly centrifuged to collect the powder at the bottom of the tube.

Reconstitute each tube in 25 µl of Milli-Q water plus 30% glycerol and store at 4°C for up to one month. To prevent bacterial growth at 4°C, add 50 µg/ml gentamicin sulfate or other antimicrobial agent. For storage longer than one month, the antibody should be aliquoted and stored at -70°C.

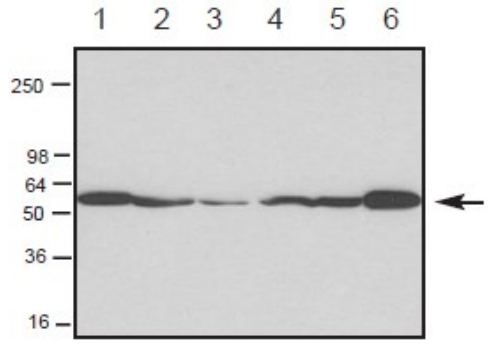
Resuspend the brain extract positive control protein in 500 µl of 1x SDS-PAGE sample loading buffer for a final concentration of 2 mg/ml, aliquot into 20 X 25 µl amounts (50 µg each), and store at -20°C.

Applications

Western Blot (WB) Applications
Use as indicated in method at 1:1000 dilution, sufficient for 25 ml of working strength Ab.

Western Blot Method:
1. Run protein samples and control samples on SDS-PAGE.
2. Equilibrate the gel in Western blot buffer (25 mM Tris pH 8.3, 192 mM glycine, 5% methanol) for 15 min at room temperature prior to electro-blotting.
3. Transfer the protein to a PVDF membrane for 60 minutes at 75V or overnight at 20V at 4°C.
4. Wash the membrane once with TBST for 10 minutes (10 mM Tris-HCl pH 8.0, 150 mM NaCl, 0.05% Tween 20).
5. Block the membrane surface with 5% nonfat-dry milk in TBST for 30 min at room temperature with constant agitation.
6. Incubate the membrane with a 1:1000 dilution of ATN02 antibody diluted in TBST/1% non-fat milk for 1h at room temperature or overnight at 4°C with constant agitation.
7. Wash the membrane 3 times in TBST for 5 min each.
8. Incubate the membrane with an appropriate dilution (e.g., 1:20,000) of anti-sheep secondary antibody in TBST/1% non-fat milk for 60 min at room temperature.
9. Wash the membrane 6 times in TBST for 10 min each.
10. Use an enhanced chemiluminescence detection method to detect the signal (e.g., SuperSignal West Dura Extended Duration Substrate; ThermoFisher).

Figure 1: Western Blot: Cross Species Reactivity of ATN02



Legend: Cell or tissue lysates (50 µg per lane) prepared in RIPA buffer were run on SDS-PAGE gels as follows; Lane 1, Drosophila S2 cells. Lane 2, Xenopus A6 cells. Lane 3, mouse Swiss 3T3 cells . Lane 4, rat NRK cells. Lane 5, human HeLa cells. Lane 6, bovine brain tissue. Proteins were transferred to PVDF membranes and probed with ATN02 at 1:1000 dilution in TBST/1% milk, a secondary antibody was used at a 1:20,000 dilution in TBST/1% milk. Blots were developed by chemilluminescence.

Immunofluorescence (IF) Applications
Use as indicated below at 1:500 dilution, sufficient for 12.5 ml of working strength Ab, approx. 125 IF slides.

IF Method
1. Grow tissue culture cells on glass coverslips to desired confluency.
2. Remove culture media and gently wash the cells once with isotemp PBS (37°C).
3. Fix the cells with methanol at -20°C for 3 min.
4. Wash the cells three times with PBS.
5. Place the coverslips with the cell side up on parafilm inside of a petri dish. Maintain a humid atmosphere by placing a piece of wet filter paper inside the covered petri dish. Add 100 µl of Permeabilization Buffer (1% Triton X-100 in PBS) to each coverslip and incubate for 20 min.
6. Remove Permeabilization Buffer, add 100 µl Block Buffer (3% BSA in PBS), and incubate for 30 min.
7. Wash the coverslips once with PBS.
8. Add 100-200 µl of a 1:500 dilution of ATN02 antibody in Blocking Buffer to each coverslip.
9. Wash each coverslip three times in Permeabilization Buffer (incubate for 5 min each).
10. Add 200 µl of a 1:500 dilution of rhodamine-conjugated anti-sheep antibody in Blocking Buffer to each coverslip. Incubate for 30 min.
11. Wash each coverslip three times in PBS (let stand for 5 min each).
12. Counterstain the DNA for 5 min with 200 µl of 100 nM DAPI in PBS.
13. Invert the coverslips on a drop of antifade mounting media on a glass slide. Gently remove the excess media with a tissue and allow mounting media to dry.
14. Examine the stained coverslips using a fluoresence microscope equipped with filter sets suitable for rhodamine and DAPI fluorophores.
15. Store the slides in the dark at 4°C.
16. Typical results are shown in Figures 2 - 4.

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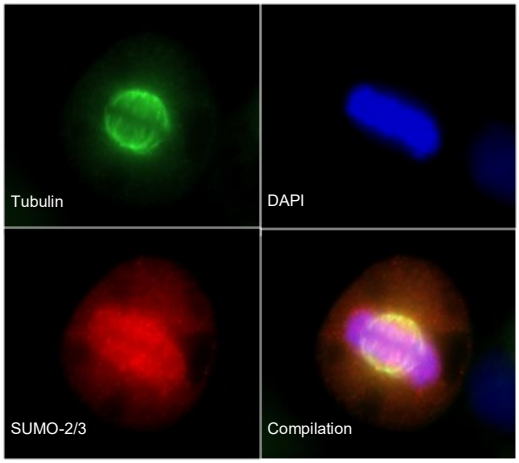
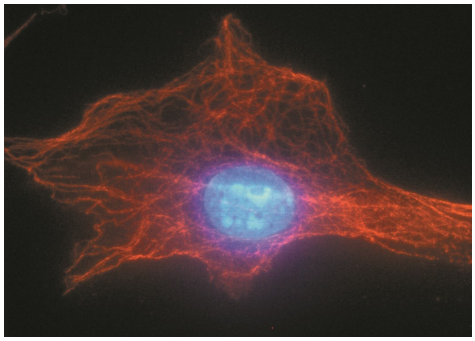
Figure 2: IF detection of microtubules, SUMOylated proteins and DNA in 3T3 Cells



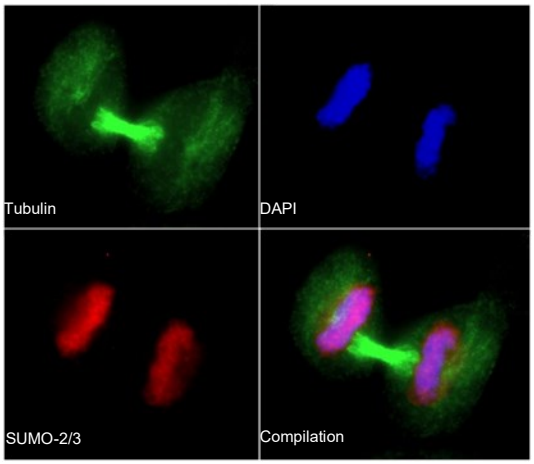
Legend: Swiss 3T3 cells were stained for microtubules (ATN02, green), DNA (DAPI, blue) and SUMOylated proteins (SUMO-2/3 Ab Cat # ASM23, red). In this experiment cells were permeabilize by incubating them with 3ml of digitonin solution (20mM HEPES pH 7.4, 110 mM Potassium acetate, 2 mM Magnesium acetate, 1X protease inhibitor cocktail, 10 mM NEM and 40 ug/mL digitonin) for 1 min with gentle agitation at room temperature. Cells were washed briefly with PBS and fixed with 4% paraformaldehyde solution for 10min at room temperature. Cells were washed in PBS and blocked with PBS/3% BSA for 30 minutes. Antibodies and DAPI were added at 1:500 dilution (ATN02, tubulin), 1: 500 dilution (ASM23, SUMO-2/3) and to 100 nM (DAPI) and incubated at room temperature for 45 minutes. Cells were washed with PBS and slides were mounted and imaged with a Zeiss Axio Observer.Z1 microscope (1.4 NA 63X objective). NOTE: the permeabilization and fixation conditions described in this experiment are not optimal for observing microtubulues (see IF method in this data sheet) but were necessary to observe SUMOylated proteins. Clear microtubule spindles are observed under these conditions.

Figure 3: IF detection of microtubules in Swiss 3T3 Cells



Legend: Swiss 3T3 cells were stained for microtubules (ATN02, red) and DNA (DAPI, blue). Cells were stained according to the method described in this data sheet and were imaged with a Zeiss Axio Observer.Z1 microscope (1.4 NA 63X objective).

Figure 4: IF detection of microtubules, SUMOylated proteins and DNA in 3T3 Cells



Legend: Swiss 3T3 cells were stained for microtubules (ATN02, green), DNA (DAPI, blue) and SUMOylated proteins (SUMO-2/3 Ab Cat # ASM23, red). In this experiment cells were permeabilize by incubating them with 3ml of digitonin solution (20mM HEPES pH 7.4, 110 mM Potassium acetate, 2 mM Magnesium acetate, 1X protease inhibitor cocktail, 10 mM NEM and 40 ug/mL digitonin) for 1 min with gentle agitation at room temperature. Cells were washed briefly with PBS and fixed with 4% paraformaldehyde solution for 10min at room temperature. Cells were washed in PBS and blocked with PBS/3% BSA for 30 minutes. Antibodies and DAPI were added at 1:500 dilution (ATN02, tubulin), 1: 500 dilution (ASM23, SUMO-2/3) and to 100 nM (DAPI) and incubated at room temperature for 45 minutes. Cells were washed with PBS and slides were mounted and imaged with a Zeiss Axio Observer.Z1 microscope (1.4 NA 63X objective). NOTE: the permeabilization and fixation conditions described in this experiment are not optimal for observing microtubulues (see IF method in this data sheet) but were necessary to observe SUMOylated proteins.

References

1. Amos, LA. & Klug A. 1974. *J. Cell Sci.* **14**: 523-530.
2. Ho C. et al. 2011. Augmin plays a critical role in organizing the spindle and phragmoplast microtubule arrays in Arabidopsis. *Plant Cell.* **23**: 2606-2618.
3. Parrotta et al. 2010. Changes in the accumulation of alpha and beta tubulin during bud development in Vitis vinifera L. *Planta* **231**: 277-291.
4. Sanbe, A et al. 2004. Desmin related cardiomyopathy in transgenic mice: a cardiac amyloidosis. *Proc. Natl. Acad. Sci.* **101**: 10132-10136.

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