

PROTOCOLS FOR DETECTION OF YAP / TAZ

WESTERN BLOT

ANTIBODIES

anti YAP/TAZ mouse monoclonal Santa Cruz sc- 101199; 1:1000
anti-TAZ (anti-WWTR1 Atlas Antibodies Sigma *Prestig*); 1:500

YAP band ~70 KDa (two bands in human lysates, one from mouse lysates)
TAZ band ~ 50 KDa

ANTI-YAP IMMUNOFLUORESCENCE

(Dupont et al., Nature 2011; Azzolin et al., 2014)

HARVESTING

- Remove medium
- wash in PBS @RT quickly (in & out in coplin jar)

FIX

- fix 10 min @RT in 4% PFA in PBS
- 2x wash in PBS @RT 3min (coplin jar)
- you can stop here: dry and store @ -80°C

Note: these two washes are absolutely essential, even if you stop & store before permeabilization)

PERMEABILIZATION

- permeabilize 10min @RT in 0.3% Triton in PBS (coplin jar)
- 3X wash, 1min each @RT in PBS (coplin jar)

BLOCKING

- block >1h @RT in 10% Goat Serum (Invitrogen, decomplexed) in PBS 0,1% Triton (PBST)

ANTIBODIES FOR YAP

anti YAP/TAZ mouse monoclonal Santa Cruz sc- 101199; 1:200
anti YAP-only mouse monoclonal Santa Cruz sc-271104, 1:200
anti YAP-only rabbit polyclonal Cell Signaling 4912S; 1:100

PRIMARY ANTIBODY

- Incubate the primary antibody (see above) in 2% Goat Serum PBST, in humid chamber overnight @4°C

WASHING

- drain the primary antibody
- - 4X washes in PBST (1min, 2min, 3min, 6min) (coplin jar)

SECONDARY ANTIBODY

- Dilute secondary antibody 1:200 in 2% GS for Alexa Secondary Antibody in the dark
- Incubate secondary antibody 1h and 30min @RT in the dark (in humid Chamber)
- 4X wash in PBST 3min/each (coplin jar)

NUCLEAR STAINING

- Incubate with DAPI or Hoechst
- 3X wash 3min in PBS @RT (coplin jar)

MOUNTING/PHOTOS

- Mount in 80% glycerol in PBS; or Mowiol for confocal.
- Take pictures
- Store slides @4°C

Note: the signal of the sc-101199 monoclonal may be low when seen on a standard IF microscope; however, the signal is clear at the confocal. For other details see Dupont et al., Nature 2011.

MODIFICATIONS FOR anti-TAZ IMMUNOFLOURESCENCE

BLOCKING

- block >1h @RT in 10% BSA (Sigma, Fraction V) in PBS 0,1% Triton (PBST)

ANTIBODIES

anti TAZ BD-Biosciences Mouse Monoclonal 560235; 1:200

PRIMARY ANTIBODY

- Incubate the primary antibody (see above) in 2% BSA in PBST, in humid chamber overnight @4°C

Note: TAZ signal is nuclear in cells cultured at low confluence on stiff substrates (i.e. plastics); on soft matrices or in cells cultured for 2 days at high-confluency, the signal disappears (correlating with TAZ protein degradation as seen by western blot; this differs from YAP which appears mainly regulated by cytoplasmic relocalization).

SECONDARY ANTIBODY

- Dilute secondary antibody 1:200 in 2% BSA for anti-mouse Alexa Secondary Antibody (i.e., goat anti-mouse 488) in the dark

ANTI-TAZ IMMUNOHISTOCHEMISTRY (Cordenonsi et al., 2011)

Immunohistochemical staining of breast cancer samples have been performed on formalin-fixed, paraffin-embedded tissue sections using a fully automated system (Bond-maX; Leica, Newcastle Upon Tyne, UK). In brief, one 4- micron-thick section from each paraffin-embedded block was cut. The sections were deparaffinized in Bond Dewax Solution (Leica) at 72°C, rinsed in ethanol, and rehydrated in distilled water. Antigen retrieval was performed by heating sections for 30 minutes at 99°C in Bond Epitope Retrieval Solution 1 (Leica). Endogenous peroxidase was blocked by 3.0% hydrogen peroxide before 30 minutes of incubation with rabbit polyclonal anti-WWTR1 (Atlas Antibodies, Sigma-Prestige = TAZ: anti-WWTR1 rabbit polyclonal Sigma, HPA007415; 1:50 diluted). Specimens were then washed with phosphate-buffered saline (PBS) and incubated with Bond Polymer Refine Detection Kit (Leica) according with the manufacturer's protocols. The staining was visualized with 3,3'-diaminobenzidine (DAB) and the slides were counterstained with Mayer's hematoxylin. The sections were then dehydrated, cleared, and mounted. Formalin-fixed, paraffin-embedded positive and negative controls were included in each run.

Note. We have noticed that the quality of the fixation is relevant for TAZ detection. Best res Tumors are fixed immediately after surgery and with abundant fixative (i.e., 10% formaline; 24 hours; 10x the volume of the sample). We have been unable to retrieve TAZ in old samples fixed with Buoin's.

IMMUNOFLUORESCENCE ON PARAFFIN SECTIONS

DAY 1

DEPARAFFINIZE SECTIONS

- 30' in 100% Xylene
- 5' in 100% Ethanol
- 5' in 95% Ethanol
- 5' in 70% Ethanol
- 5' in H₂O

ANTIGEN RETRIEVAL

- Place the slide rack into a plastic container and cover the slides with Citrate Buffer pH6; cover the wash container with an appropriate lid. Note: Citrate Buffer (500 ml): 1,47g Sodium Citrate Dihydrate, dH₂O 500 ml. Mix to dissolve. Adjust pH to 6 then add 2,5 ml Tween 10% and mix well. Store at RT.
- Heat in microwave until boiling start (about 3/4 minutes).
- Reduce the power to 20% for 10'.
- Remove from microwave and allow to cool at RT (you can add some cytrate buffer to accelerate the process).

PERMEABILIZATION

- Wash (in/out) in PBS
- permeabilize 20 min @RT in 1% Triton in PBS
- 3X wash, 1 min each @RT in PBS

BLOCKING

- block 1h @RT in 10% Goat Serum (Invitrogen, decomplexed) in PBS 0,1% Triton (PBST)

PRIMARY ANTIBODY

- Incubate the primary antibody in 2% Goat Serum PBST, in humid chamber overnight @4°C

DAY 2

WASHING

- drain the primary antibody
- - 4X washes in PBST (1min, 2min, 3min, 6min) (coplin jar)

SECONDARY ANTIBODY

- Dilute secondary antibody 1:200 in 2% GS for Alexa Secondary Antibody in the dark
- Incubate secondary antibody 1h and 30min @RT in the dark (in humid Chamber)
- 4X wash in PBST 3min/each (coplin jar)

NUCLEAR STAINING

- Incubate with DAPI or Hoechst, 30 min
- 3X wash 3min in PBS @RT (coplin jar)

MOUNTING/PHOTOS

- Mount in 80% glycerol in PBS; or Mowiol for confocal.
- Take pictures
- Store slides @4°C