Mouse anti-TARDBP (Novus cat. H00023435-M01)

Working protocol (based on FFPE human multiple tissues)

1. Slides de- deparaffinization and rehydration (xylene and alcohols) to distillate water
2. Antigen retrieval (microwave in citrate buffer Ph6 for 20 minutes, cool down for another 20 minutes). Alternatively incubate in citrate buffer Ph6 overnight at 70°C (when tissue is easy to be displaced from the slides)
3. Wash in distillate water 3 × 5 minutes
4. Endogenous peroxidase block in 3% water peroxide for 30 minutes
5. Wash in distillate water 3 × 5 minutes
6. Wash in 1×PBS for 3 × 5 minutes
7. Blocking unspecific antigenic sites in 3% skim milk in 1×PBS for 30 minutes
8. Tap of the skim milk solution
9. Without further washing, add 200 µl of primary antibody per slide (Novus anti-TARDBP , code **H00023435-M01**, diluted as 1:500 in 1% bovine serum albumin in 1×PBS). Incubate the slides at 4°C overnight.
10. Next day the slides are taken out from the fridge and let to come to room temperature
11. Thorough washing in 1×PBS for 4 × 5 minutes
12. Drop on each slide a few drops of anti-mouse, human adsorbed polymer-HRP amplification system (Histofine Simple Stain Max PO, code 414131F). Incubate for 30 minutes
13. Wash in 1×PBS for 3 × 5 minutes
14. DAB detection for less than 2 minutes (Dako Liquid DAB+ Substrate, code K3467)
15. Counterstain with haematoxylin
16. Dehydrate, and clear in xylene for at least 2 hours
17. Mount with a xylene-based mounting medium.

* For all tissue slides utilized in this review we also performed negative control staining by skipping the primary antibody.

Results – Image Interpretation

Brain - gray matter, the antibody labels both the nucleus and the cytoplasm of neurons. The signal does not seem to extend into neuritis (Pirici et al., 2011).

Brain - white matter, oligodendrocytes are not labeled.

Myometrium – the nuclei of smooth muscle cells express the antigen, but not very strong.

Striated muscle fibers express TARDBP both in the nucleus and their striations.

Mouth- mixt salivary gland, the antibody sharply labels the nuclei of excretory ducts and serous acini. Almost no expression is observed in mucous acini.

Stomach – upper gastric mucosa, the antibody labels the nuclei of gastric surface epithelium and glands; a few stromal cells (mostly monocyte-looking cells) are also presenting this signal.

Stomach – deep gastric glands, in addition to the other elements described above, the antibody shows a granular-like appearance in the principal gastric cells, most frequently toward the apical pole of the cell. This might correspond to pepsinogen secretion granules. However no reference could be found in the literature regarding the expression of TARDBP in these structures. This could be also due to cross reactivity of this polyclonal antibody with some components of the secretion granules. This signal seems much stronger than the nuclear signal in these glands.

Ileum- intestinal vilosities, the antibody labels the enterocytes' nuclei. A few stromal cells (mostly monocyte-looking cells) are also presenting this signal.

Lymph ganglia – most of the macrophage (APCs) in the clear germinal center express TARDBP, as well as the lymphocytic corona. Still, not all populations of lymphocytes seem to express TARDBP.

Pancreas. Compared to the serous component of salivary glands, TARDBP is not expressed in the nuclei of pancreatic serous acini or in the nuclei/cytoplasm of Langerhans islets. However, the signal is well present in the cytoplasm of pancreatic acinar cells.

Lung – except some monocytes – macrophages, no other component expresses TARDBP.

Kidney – cortex. The nuclei of mostly distal contort tubules express TARDBP. Some components of the glomerulus also express it but to a lower extent.

Endometrium. The nuclei of the endometrial glands' cells strongly express TARDBP. Some of the stromal cells also express it but to a lower extent.

Reference List

Pirici,D., Pirici,I., Mogoanta,L., Margaritescu,O., Tudorica,V., Margaritescu,C., Ion,D.A., Simionescu,C., and Coconu,M. (2011). Matrix metalloproteinase-9 expression in the nuclear compartment of neurons and glial cells in aging and stroke. Neuropathology.