Monoclonal antibody to TPX2 for cancer diagnosis

Protein TPX2, associated with microtubules, is a regulator of cell division. It has been shown that TPX2 is often aberrantly expressed in cancer cells and thus represents a new marker for cancer diagnosis and prognosis. New monoclonal antibody, TPX2-01, prepared in the Institute of Molecular Genetics, AS CR v.v.i., specifically recognizes human TPX2 in immunoblotting. In attempt to determine the range of the antibody applicability, TPX2-01 specificity and reactivity in the other immunological assays was determined in the frame of TACR gamma project. Epitope sequence was also determined. The results revealed that mouse monoclonal antibody TPX2-01 (IgG1, kappa) is suitable for ELISA tests (**Fig. 1A and 1B**), immunoblotting (**Fig. 2A and 2B**), and immunoprecipitation (**Fig. 2C**). Antibody labels TPX2 both in immunofluorescence microscopy (**Fig. 3a and 3b**) and on paraffin-embedded sections (**Fig. 3c**). Antibody-binding region on TPX2 molecule was determined by pulldown experiments (**Fig. 4A**). Epitope mapping with immobilized synthetic peptides led to more precise localization of the target epitope (**Fig. 4B**). Antibody TPX2-01 recognizes amino acid sequence $_{636}$EPFVPKKEKKS$_{646}$ (**Fig. 4C**), that is exposed on the surface of TPX2 molecule.
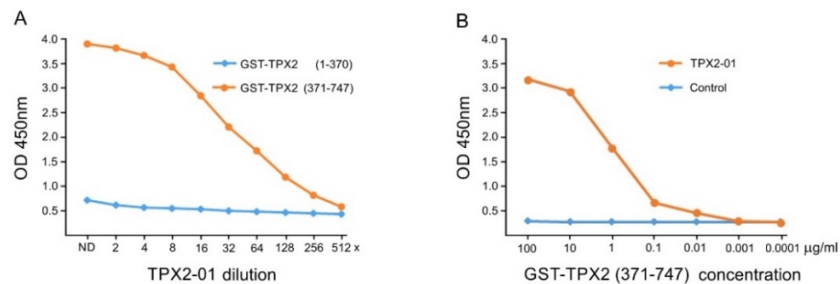

Fig. 1. ELISA tests with TPX2-01. (A) Titration of antibody in the form of hybridoma supernatant on the immobilized TPX2 protein. Domains 371-747 (containing epitope) and 1-370 (negative control) were immobilized at the concentration 20 µg/ml. ND, non-diluted antibody. (B) Detection of the immobilized GST-TPX2 protein with TPX2-01 and a negative control antibody to tubulin (Control). Antibodies were used in the form of undiluted supernatant.
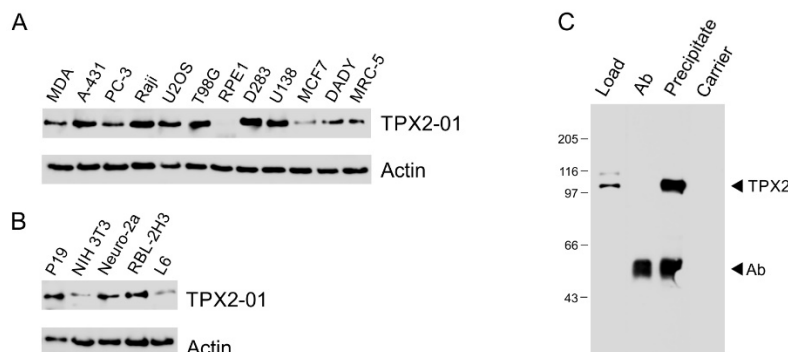

Fig. 2. Analysis of TPX2 expression in cell lines (A-B) and its immunoprecipitation (C) using TPX2-01. (A) Immunoblot analysis of total cell lysates from selected human cell lines solubilized in SDS-sample buffer. (B) Immunoblot analysis of total cell lysates from mouse (P19, NIH 3T3, Neuro-2a) and rat (RBL-2H3, L6) cell lines. Actin, loading control. (C) Immunoprecipitation from human HEK293 nuclear extract in RIPA buffer. Ab, immobilized antibody without extract; Carrier, protein G without antibody incubated with extract. TPX2-01 was used both for immunoprecipitation and immunoblotting.
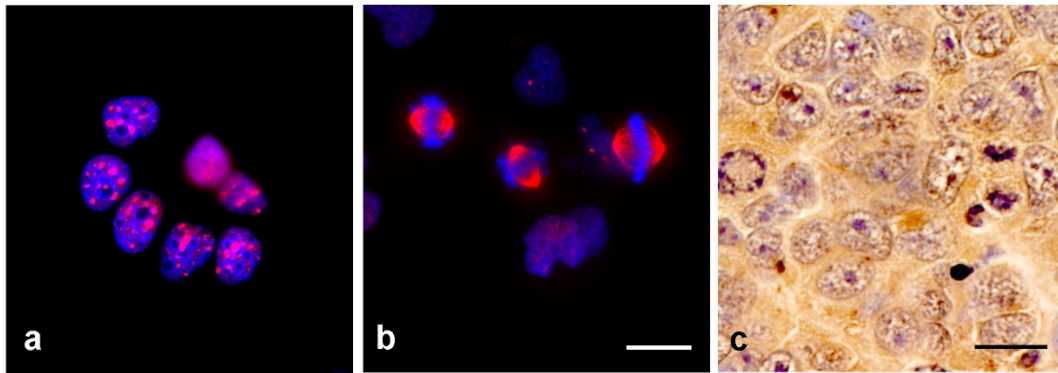
Fig. 3. Localization of TPX2 in human glioblastoma cell line T98G (a-b) and in brain tumor (c) using TPX2-01 antibody. (a-b) Indirect immunofluorescence microscopy on cells fixed by formaldehyde and extracted by Triton X-100. DNA stained with DAPI. In interphase cells, TPX2 localizes to nuclei (a), in mitosis it localizes to microtubules of mitotic spindle (b). (c) Tissue section of human glioma in mouse brain (orthotopic xenograft, human U87MG glioblastoma cells). Formaldehyde-fixed and paraffin-embedded tissue. Antibody was detected by avidin-biotin peroxidase complex and DAB. Hematoxylin nuclear counterstaining. Scale bar: 20 µm.
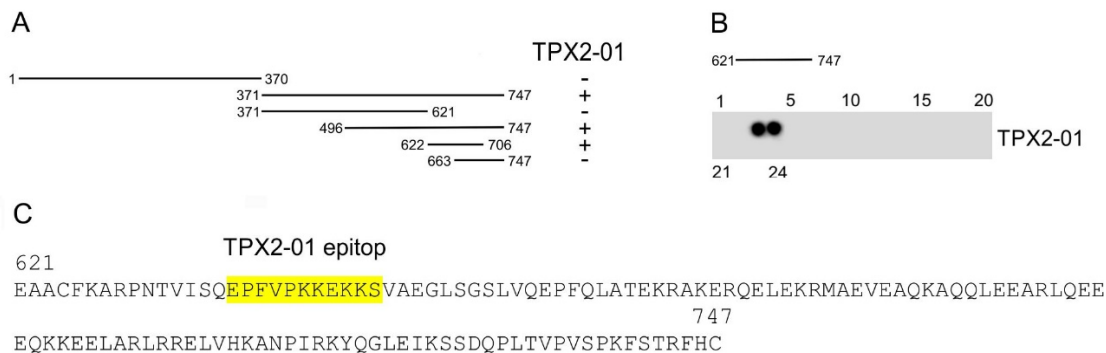

Fig. 4. Identification of the epitope recognized by TPX2-01. (A) Analysis of the antibody reactivity with GST-constructs of TPX2 protein (+, positive reaction) localized the targeted epitope to amino acid (aa) region 622-66. (B) Epitope mapping on immobilized overlapping synthetic peptides (15-mers, overlap 10 aa) covering the aa region 621-747. (C) Localization of the epitope recognized by TPX2-01 is indicated by yellow color..

Conclusions: The results show that mouse monoclonal antibody TPX2-01 (IgG1, kappa) exhibits properties, which predestine it for ELISA tests, immunoblotting, immunoprecipitation, immunocytochemical and histochemical experiments. Epitope sequence recognized by TPX2-01 was identified.

To get more information about the TPX2-01 antibody or to buy nonexclusive licence for hybridoma cells producing the antibody, please contact Center for Technology Trasfer, IMG AS CR, Videnska 1083, 14220 Praha 4, Czech Republic; Tel. (420-241 063 227 or 420-602 892 876).