ELISA test for gamma-tubulin detection in cancer diagnosis

Gamma-tubulin is a key protein of nucleation complexes that are essential for microtubule organization. It has been shown that gamma-tubulin, encoded by two genes in human, is overexpressed in cancer cells and thus represents a new marker for glioma cancer prognosis. Some microtubule proteins can be detected in body fluids under pathological conditions. In comparison with ELISA kits to alpha- and beta-tubulins, which are used in scientific publications, the availability of kits suitable for analysis of soluble gamma-tubulin is very limited. Determination of soluble gamma-tubulin concentration in tissue homogenenates, cell lysates, or body fluids using ELISA kits has been not reported. A panel of well-characterized monoclonal antibodies specific to gamma-tubulin was prepared at the Institute of Molecular Genetics of the ASCR, v. v. i. Within the framework of TACR Gamma project we have determined their applicability for preparation of highly sensitive ELISA test for detection of gamma-tubulin, which can be used for basic research as well as for diagnostic purposes.

Purified monoclonal antibodies were conjugated with biotin and tested in combination with commercially available antibodies against phylogenetically conserved epitopes of gamma-tubulin using a purified gamma-tubulin standard. The finding of suitable combination of antibodies allowed us to optimize conditions of the ELISA test with respect to the amount of used reagents as well as methods of signal detection and amplification. We have determined the target epitope recognized by the monoclonal antibody. Sensitivity and reproducibility of the ELISA test was verified on different types of samples. Our results show that antibodies selected against gamma-tubulin (affinity-purified rabbit polyclonal antibody for immobilization of gamma-tubulin and biotin-conjugated mouse monoclonal antibody for detection of bound gamma-tubulin) are very specific **(Fig.v1A)** and purified gamma-tubulin does not contain contaminants **(Fig. 1B)**. Sandwich ELISA enables detection of gamma-tubulin in concentrations less than 1 ng/ml **(Fig. 2)**, and it is possible to determine the gamma-tubulin concentration in complex cell lysates **(Fig. 3A)** and in body fluids. The first preliminary analysis of human blood serum from the control group and patients with cancers has shown enhanced concentration of gamma-tubulin in different types of cancer **(Fig. 3B)**. Epitope mapping with synthetic peptides revealed that monoclonal antibody recognizes a phylogenetically conserved epitope, which is present in both human gamma-tubulin genes **(Fig. 4)**. In accordance with the fact that polyclonal antibody also recognizes the phylogenetically conserved epitope, it is possible to assume that the ELISA test will be suitable for detection of gamma-tubulin in different species. An identical sequences for both antibodies was identified in human (*Homo sapiens*), mouse (*Mus musculus*), chicken (*Gallus gallus*) and frog (*Xenopus laevis*) gamma-tubulins.

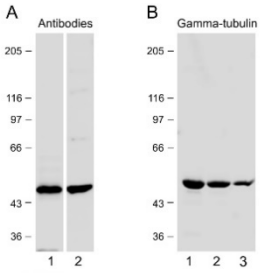

Fig. 1. Characterization of antibodies and gamma-tubulin standard. (A) Immunoblots of human cell lysates stained with antibodies against gamma-tubulin. Polyclonal antibody (1), monoclonal antibody (2). (B) Gamma-tubulin standard in concentrations 0.5 mg/ml (1), 0.25 mg/ml (2) and 0.125 mg/ml (3).

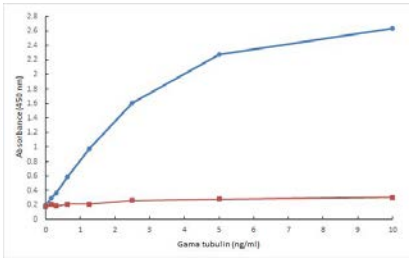

Fig. 2. Detection of different concentrations of gamma-tubulin using the ELISA test. Gamma-tubulin (blue line), control without immobilizing antibody (red line).

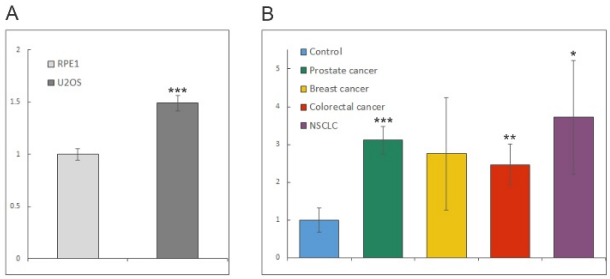

Fig. 3. Comparison of gamma-tubulin concentrations in cell lysates and blood sera. (A) Relative comparison of gamma-tubulin concentrations in 1% NP40 lysates of cell lines RPE1 (human retina) and U2OS (human osteosarcoma) (n=3). (B) Relative comparison of gamma-tubulin concentrations in blood serum of healthy persons (Control) and patients with different types of cancer (n=4). *$*p < 0.05$, $**p < 0.01$, $***p < 0.001$*

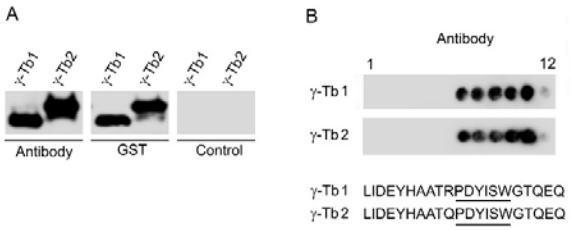

Fig. 4. Identification of the epitope recognized by monoclonal antibody to gamma-tubulin. (A) Immunoblots of immobilized GST-tagged human gamma-tubulin isoforms (γ-Tb1 and γ-Tb2) with monoclonal antibody to gamma-tubulin (Antibody) and antibody to GST. In control, we used secondary anti-mouse antibody (Control). (B) Immobilized overlapping synthetic peptides of both human gamma-tubulin isoforms (γ-Tb1 and γ-Tb2) stained with monoclonal antibody (Antibody). Each peptide contains 10 amino acids with 9 amino acid overlap, numbers mark positions of peptides.

Conclusion: The results show that the sensitive ELISA test for detection of gamma-tubulin has properties that predict its usage in quantification of gamma-tubulin in different types of samples. Potentially, the ELISA test could also be used in cancer diagnosis. As antibodies to gamma-tubulin recognize phylogenetically highly conserved epitopes, it is expected that such test can be applied for quantification of gamma-tubulin of different species.

To get more information about the ELISA test or to purchase a non-exclusive licence for 1/ hybridoma cells producing mouse monoclonal antibody, 2/ plasmid encoding gamma-tubulin and 3/ know-how to perform the ELISA test, please, contact the Centre for Transfer Technology, IMG AS CR, Vídeňská 1083, 14220 Prague 4, Czech Republic (tel. +420-241 063 227 or +420-602 892 876).