Get Focused

Issue 1

EXBIO Antibodies

TUBULINS

The **microtubules** are intracellular dynamic polymers made up of evolutionarily conserved polymorphic **alpha/beta-tubulin heterodimers** and a large number of **microtubule-associated proteins (MAPs)**.

*The microtubules consist of 13 protofilaments and have an outer diameter 25 nm. Microtubules have their intrinsic polarity and comprise highly dynamic plus ends and less dynamic minus ends. Microtubules are required for vital processes in eukaryotic cells including mitosis, meiosis, maintenance of cell shape and intracellular transport. Microtubules are also necessary for movement of cells by means of flagella and cilia. In mammalian tissue culture cells microtubules have their minus ends anchored in microtubule organizing centers (**MTOCs**).The **GTP** (guanosintriphosphate) molecule is an essential for tubulin heterodimer to associate with other heterodimers to form microtubule. In vivo, microtubule dynamics vary considerably. Microtubule polymerization is reversible and a populations of microtubules in cells are on their minus ends either growing or shortening – this phenomenon is called **dynamic instability of microtubules**. On a practical level, microtubules can easily be stabilized by the addition of non-hydrolysable analogues of GTP (eg. **GMPPCP**) or more commonly by anti-cancer drugs such as **Taxol**. Taxol stabilizes microtubules at room temperature for many hours. Using limited proteolysis by enzymes both tubulin subunits can be divided into N-terminal and C-terminal **structural domains**.*

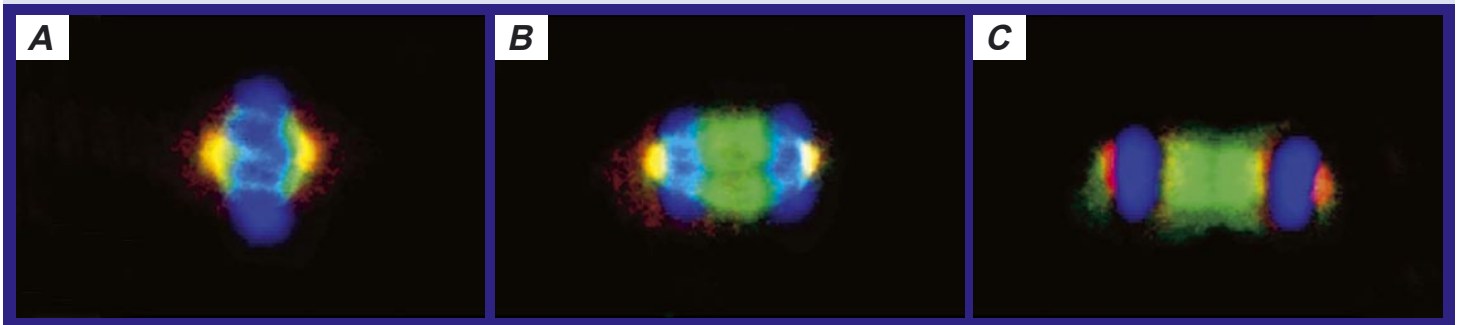

Fig. 1. Immunofluorescence staining of 3T3 mouse embryonal fibroblast cell line – metaphase (A), anaphase (B) and telophase (C).
Tubulin stained with polyclonal antibody (green), gamma-tubulin stained with antibody TU-30 (red), DNA (blue).
Author: E. Dráberová et al., Laboratory of the Biology of Cytoskeleton, Institute of Molecular Genetics, Academy of the Sciences, Prague, Czech Republic.

The alpha-, beta- and gamma-tubulins are homologous and belong to a tubulin family:

The **alpha-tubulin** (relative molecular weight about 50 kDa) is globular protein that exists in cells as part of soluble alpha/beta-tubulin dimer or it is polymerized into microtubules. In different species it is coded by multiple tubulin genes that form **tubulin classes** (in human 6 genes). Expressed tubulin genes are named **tubulin isotypes**. Some of the tubulin isotypes are expressed ubiquitously, while some have more restricted tissue expression. Alpha-tubulin is also subject of numerous post-translational modifications. Tubulin isotypes and their posttranslational modifications are responsible for multiple tubulin charge variants – **tubulin isoforms.** Heterogeneity of alpha-tubulin is concentrated in C-terminal structural domain.

The **beta-tubulin** (relative molecular weight about 50 kDa) is counterpart of alpha-tubulin in tubulin heterodimer, it is coded by multiple tubulin genes and it is also posttranslationally modified. Heterogeneity of subunit is concentrated in C-terminal structural domain.

The **class III beta-tubulin** isotype is present dominantly in cells of neuronal origin and it is one of the earliest marker of neuronal differentiation. Class III beta-tubulin, is regarded as a specific probe for the cells of neuronal origin as well as for the tumours originating from these cells. The neuron-associated class III beta-tubulin isotype is most abundant in cells of neuronal origin but was also detected in Sertoli cells of the testis and transiently in non-neuronal embryonic tissues.

The **gamma-tubulin** (relative molecular weight about 48 kDa) is minor member of tubulin family (less that 0.01% of tubulin dimer). The gamma-tubulin is coded only by two genes, but it is also posttranslationally modified. In mammalian interphase cells it is primarly localized in MTOCs in perinuclear region, in mitotic cells it is on spindle pole bodies.

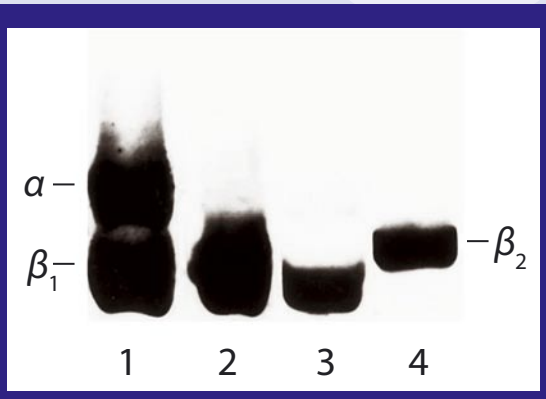

Fig. 2. Reactivity of monoclonal antibodies with reduced and carboxymethylated porcine brain tubulin.
Immunoblot with monoclonal antibodies. Lanes 1-4 immunoreactivity with antibodies TU-18, TU-12, TU-14 and TU-20, respectively; 30 µg protein per lane; 7,5% SDS-polyacrylamide gel. β_1 and β_2 denote the electrophoretic mobility of the major and minor (class III) β-tubulin components, respectively.

Draberova E, Lukas Z, Ivanyi D, Viklicky V, Draber P.: Expression of class III beta-tubulin in normal and neoplastic human tissues. Histochem Cell Biol. 1998 Mar;109(3):231-9.

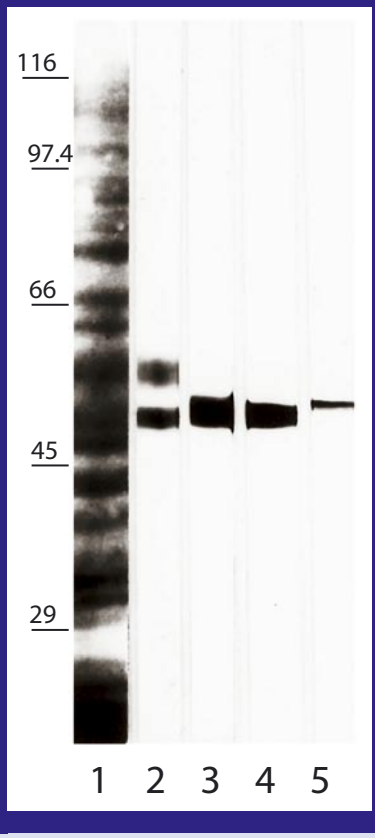

Fig. 3. Specifity of antibodies determined by immunoblotting.
Immunoblot of total extract of porcine brain with anti-tubulin monoclonal antibodies. Lane 1 amido black staining of transferred proteins. Lanes 2-5 immunoreactivity with antibodies TU-18, TU-12, TU-14 and TU-20, respectively; 5-15% gradient SDS-polyacrylamide gel. Bars on the left margine indicate positions, from top to bottom, of specific molecular weight markers (116, 97.4, 66, 45 and 29 kDa).

Draberova E, Lukas Z, Ivanyi D, Viklicky V, Draber P.: Expression of class III beta-tubulin in normal and neoplastic human tissues. Histochem Cell Biol. 1998 Mar;109(3):231-9.

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MONOCLONAL ANTIBODIES

The monoclonal antibodies to tubulins were developed and characterized by group of **Pavel Dráber, PhD. in Laboratory of the Biology of Cytoskeleton,** Institute of Molecular Genetics, Academy of the Sciences, Prague, Czech Republic.

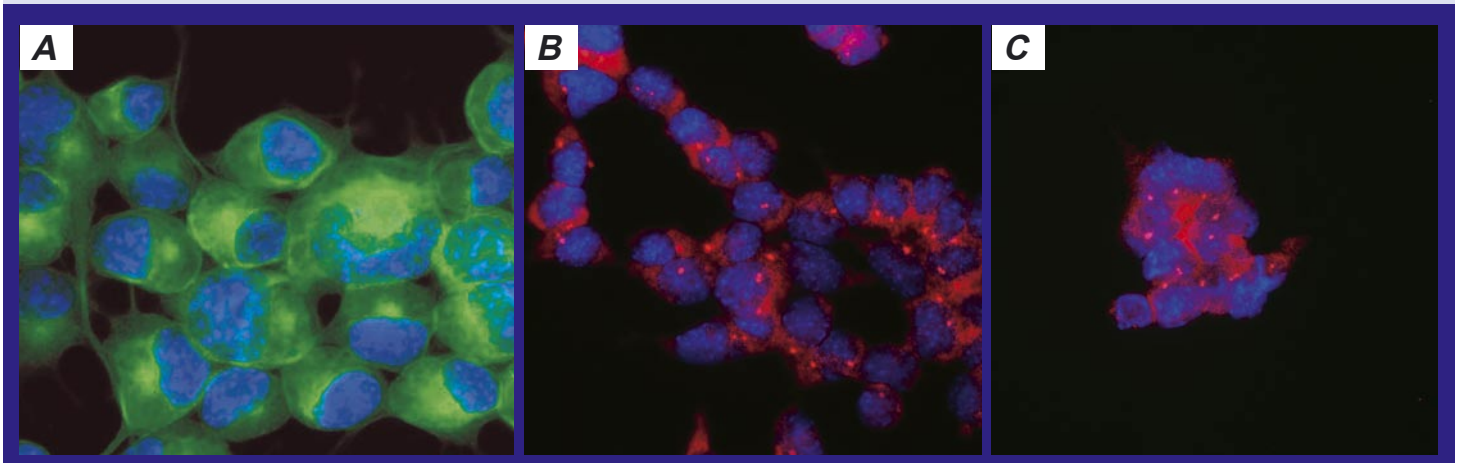

Fig. 4. Immunofluorescence staining with Mouse Monoclonal Antibodies to Tubulin.

A *– direct conjugate TU-20/FITC to betaIII-tubulin (green), DNA (blue), Neuro2a mouse neuroblastoma cell line;*
B *- TU-30 to gamma-tubulin (red; dilution of primary antibody 1-2 µg/ml, detection by secondary antibody GAM/Cy3 conjugate), DNA (blue), P19X1 mouse embryonal carcinoma cell line;*
C *– direct conjugate TU-30/Dyomics 547 to gamma-tubulin (red; dilution 2-4 µg/ml), DNA (blue), P19X1 mouse embryonal carcinoma cell line.*

Author: E. Dráberová et al., Laboratory of the Biology of Cytoskeleton, Institute of Molecular Genetics, Academy of the Sciences, Prague, Czech Republic.

Antibodies to alpha/beta-tubulin dimer:

CLONE ISOTYPE	SPECIFIC DOMAIN	REF. Nr.	WB (reducing conditions)	ICC		
				Fixed	Unfixed	In living cells
TU-08 IgM	alpha,beta-tubulin dimer	9	+	+		
TU-10 IgM	alpha,beta-tubulin dimer		+	+		

Antibodies to alpha-tubulin:

CLONE ISOTYPE	SPECIFIC DOMAIN	MAPPED EPITOPE	REF. Nr.	WB (reducing conditions)	IP	ICC		
						Fixed	Unfixed	In living cells
TU-01 IgG1	N-terminal structural domain	aa 65-97	1,2,3,4,5,18	+	-	+	-	-
TU-02 IgM	N-terminal structural domain		3,6	+	-	+	-	-
TU-04 IgM	C-terminal structural domain		3,5,8	+	-	+	-	-
TU-07 IgM	C-terminal structural domain		3,8	+	+	+	+	+
TU-16 IgM	alpha-tubulin		9,19,20	+	+	+	+	

Antibodies to beta-tubulin:

CLONE ISOTYPE	SPECIFIC DOMAIN	MAPPED EPITOPE	REF. Nr.	WB (reducing conditions)	ICC		
					Fixed	Unfixed	In living cells
TU-06 IgM	N-terminal structural domain	aa 81-95	3,4,5,11,18, 21	+	+	-	-
TU-11 IgM	C-terminal structural domain		3,4	+	+	+	+
TU-12 IgM	beta-Tubulin	aa 345-430	8,12	+	+	+	+
TU-13 IgM	N-terminal structural domain		7,11	+	+	-	-
TU-14 IgM	C-terminal structural domain	aa 431-444	3,4,8	+	+	+	+

Antibody to betaIII-tubulin (class III beta-tubulin):

The **TU-20** monoclonal antibody recognizes the peptide sequence ESESQGPK (aa 441-448 - C-terminal end) of human class III beta-tubulin specific for neurones. The class III beta-tubulin isotype is widely used as a neuronal marker in normal and neoplastic tissues (Dráberová E. et al., 1998). Class III beta-tubulin antibodies are commonly used as neuronal markers.

TU-20 is recommended for use in Western Blotting, Immunohistochemistry (paraffin sections) and Immunocytochemistry.

CLONE ISOTYPE	SPECIFIC DOMAIN	MAPPED EPITOPE	REF. Nr.	WB (reducing conditions)	IHC	ICC		
						Fixed	Unfixed	In living cells
TU-20 IgG1b	betaIII-tubulin	aa 441-448	11,13,22	+	+ (paraffin)	+	-	-

Antibodies to gamma-tubulin:

The **TU-30** monoclonal antibody is an excellent antibody recognizes C-terminal peptid EYHAATRPDYISWGTQ of gamma-tubulin. The antibody decorates interphase in mammalian cells centrosomes as well as half-spindles and midbodies in mitotic cells of various origin (Nováková et al., 1996). **TU-30** is recommended for use in immunocytochemistry (excellent) and Western Blotting.

CLONE ISOTYPE	SPECIFIC DOMAIN	MAPPED EPITOPE	REF. Nr.	WB (reducing conditions)	ICC		
					Fixed	Unfixed	In living cells
TU-30 IgG2b	gamma-tubulin	aa 434-449	14,16,17,19,21	+	+	-	-

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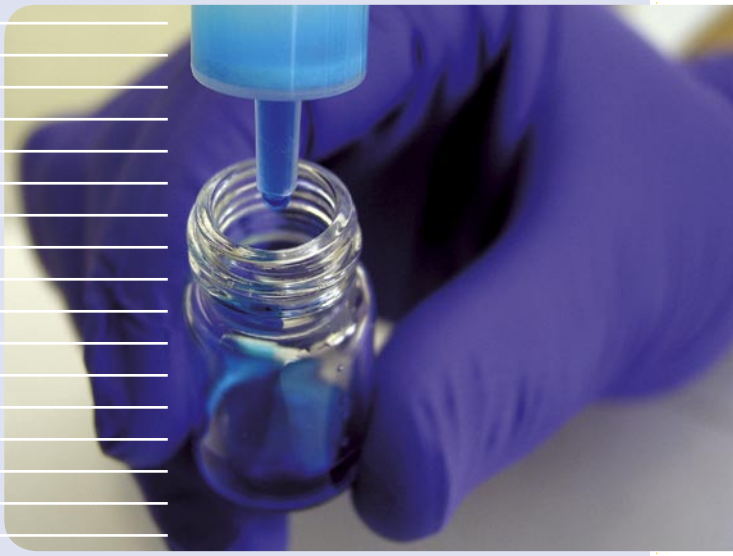

Cytoskeleton - Microtubule and Associated Proteins in EXBIO Offer:

Specifity	Clone	Host/ Isotype	Reactivity	Application	Catalogue Nr.	Formats
alpha, beta-tubulin dimer	TU-08	Ms/IgM	Hu, Por, Ms	WB, ICC	11-445-R100	purified (0.1 ml)
alpha, beta-tubulin dimer	TU-10	Ms/IgM	All Species	WB, ICC	11-446-C100	purified (0.1 mg)
alpha-tubulin	TU-01	Ms/IgG1	All Species	WB, ICC	11-250-C100	purified (0.1 mg)
					11-250-M001	purified (1.0 mg)
					1F-250-C100	FITC (0.1 mg)
					1C-250-C100	DY547 (0.1 mg)
alpha-tubulin	TU-02	Ms/IgM	Hu, Por, Ms	WB	11-251-C100	purified (0.1 mg)
					11-251-M001	purified (1.0 mg)
alpha-tubulin	TU-16	Ms/IgM	All Species	IP, WB, ICC, ELISA	11-283-C100	purified (0.1 mg)
					11-283-M001	purified (1.0 mg)
beta-tubulin	TU-06	Ms/IgM	All Species	WB, ICC	11-251-C100	purified (0.1 mg)
					11-251-M001	purified (1.0 mg)
beta-tubulin	TU-11	Ms/IgM	Por, Ms	WB	11-448-C100	purified (0.1 mg)
					11-448-M001	purified (1.0 mg)
beta-tubulin	TU-12	Ms/IgM	All Species	WB, ICC	11-427-C100	purified (0.1 mg)
					1C-427-C100	DY547 (0.1 mg)
betaIII-tubulin	TU-20	Ms/IgG1	All Species	WB, IHC(P), ICC	11-264-C100	purified (0.1mg)
					11-264-M001	purified (1.0 mg)
					1F-264-C100	FITC (0.1 mg)
gamma-tubulin	TU-30	Ms/IgG2b	Hu, Por, Ms, Rt, Chk, Prot, Plants	ICC, WB	11-465-C100	purified (0.1 mg)
					11-465-M001	purified (1.0 mg)
					1C-465-C100	DY547 (0.1 mg)
					1D-465-C100	DY647 (0.1 mg)
Kinesin	polyclonal	Rb	Hu, Por, Ms	WB, ICC	11-434-C100	purified (0.1 mg)
Kinesin (heavy chain)	KN-01	Ms/IgM	Hu, Por, Ms	ICC, ELISA	11-350-C100	purified (0.1 mg)
Kinesin (heavy chain)	KN-02	Ms/IgM	Hu, Por, Ms, Rt	ICC	11-390-C100	purified (0.1 mg)
Kinesin (heavy chain)	KN-03	Ms/IgM	Hu, Por, Ms, Rt	ICC	11-391-C100	purified (0.1 mg)
Microtubule Associated Protein 2ab	MT-07	Ms/IgG1	Hu, Por, Ms	IP, WB, IHC(F), ICC, ELISA	11-472-C100	purified (0.1 mg)
Microtubule Associated Protein 2ab	MT-08	Ms/IgG1	Hu, Por, Ms	IP, WB, IHC(F), ICC, ELISA	11-473-C100	purified (0.1 mg)
Microtubule Associated Protein 2ab	MT-01	Ms/IgG1	Por, Ms, Bv, Fel	IP, WB, ICC	11-253-C100	purified (0.1 mg)
					11-253-M001	purified (1.0 mg)

Bv = Bovine; Chk = Chicken; Fel = Feline (Cat); Hu = Human; Ms = Mouse; Por = Porcine; Prot = Protozoa; Rb = Rabbit; Rt = Rat