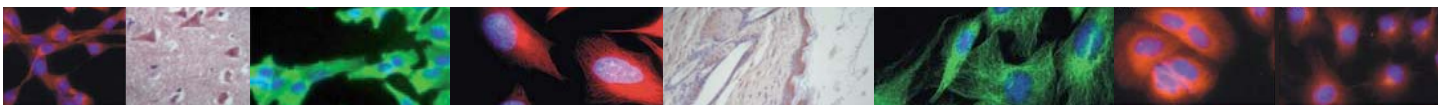


## Antibodies to Loading Controls



Western blot analysis can be used for (semi-) quantitative detection of target proteins, especially concerning experimental procedures which lead to altered expression levels of the target protein.

To compare the effect of different treatments on the target protein a method to standardize the amount of total protein loaded per lane and to confirm that equal amounts were loaded in each lane of the gel is needed.

For this purpose so called 'house keeping' proteins are selected as controls. The loading control ideally should not show an altered expression level during the course of the experiment so one has to carefully choose the appropriate loading control for each experimental setup. Proteins selected as loading controls are often of fundamental importance in maintaining basic cellular functions.

### Actin beta

In vertebrates 3 main groups of actin isoforms - alpha, beta and gamma - have been identified. The alpha actins are found in muscle tissues and are a major constituent of the contractile apparatus. The beta and gamma actins coexist in most cell types as components of the cytoskeleton and as mediators of internal cell motility. **Beta actin (ACTB)** is a highly conserved protein that is involved in cell motility, structure and integrity. Beta actin is a cytoplasmic protein ubiquitously expressed in all eukaryotic cells and therefore often used as a loading control.

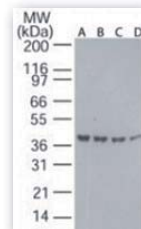
### Tubulin alpha and beta

Heterodimers consisting of alpha- and beta-tubulin form microtubules of the eukaryotic cytoskeleton. **Tubulin beta5 (TUBB5)** and **tubulin alpha (1B chain/TUBA1B)** are ubiquitously expressed representing a good choice as loading controls for cells of multiple origins.

**Class III beta tubulin (TUBB3/TUBB4)** is a vertebrate tubulin isotype only found in neurons. Exception to the neuro-specificity of beta III tubulin occurs in cells in the mammalian testis during cancer development. Therefore, TUBB3 can be used as a loading control for neuronal samples. Tubulins are generally highly conserved proteins throughout all eukaryote species. Most available antibodies to tubulins are reactive on nearly all species tested so far.

### GAPDH

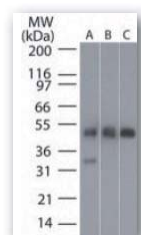
**Glyceraldehyde 3 phosphate dehydrogenase (GAPDH)** is well known as one of the key enzymes involved in glycolysis. GAPDH, which is constitutively expressed in almost all tissues at high levels, is suitable as an excellent loading control.



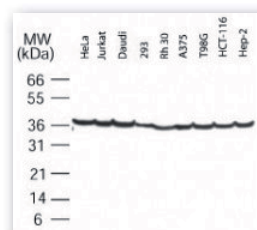
**Fig. 1:** Western blot analysis of beta actin in decreasing amounts of human ovary tissue lysate using **Cat.-No. AP21589PU-N**: A) 40 µg, B) 30 µg, C) 20 µg, and D) 10 µg per lane



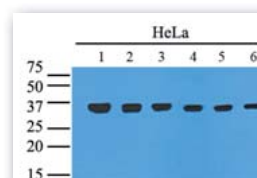
**Fig. 2:** Western blot using affinity purified alpha tubulin antibody **Cat.-No. AP09289PU-N** shows detection of a predominant band at ~50 kDa corresponding to alpha tubulin (arrowhead). Lanes correspond to whole cell lysates from mouse brain (lane 1), rat brain (lane 2), A431 cells (lane 3), Jurkat cells (lane 4) and HeLa cells (lane 5)



**Fig. 3:** Western blot analysis of beta tubulin (arrowhead) in A: human brain, B: mouse brain and C: rat brain tissue lysates using **Cat.-No. AP21644PU-N**



**Fig. 4:** Application of the GAPDH antibody as a protein loading control in Western blots. Total proteins from various human cell lysates were normalized using the GAPDH monoclonal antibody **Cat.-No. SM7116P**



**Fig. 5:** Western blot analysis: Cell lysates of HeLa were probed with GAPDH antibody **Cat.-No. AM09367PU-N**; Lane 1: 1:1,000, lane 2: 1:2,000, lane 3: 1:4,000, lane 4: 1:6,000, lane 5: 1:8,000, lane 6: 1:10,000

## VDAC

Voltage-dependent anion-selective channel protein 1 (VDAC) is an outer membrane mitochondrial protein. The VDAC proteins are thought to form aqueous channels, or pores, through which adenine nucleotides cross the outer mitochondrial membrane. VDACS have been implicated in the formation of the mitochondrial permeability transition pore complex in apoptotic cells. VDAC is highly expressed in heart, liver and skeletal muscle, where concentrations of mitochondria are at their highest. VDAC can be used as a loading control with mitochondrial preparations as well as whole cell lysates.

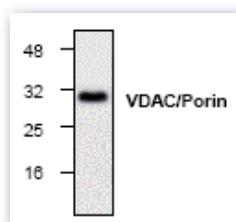


Fig. 6: Western blot analysis of VDAC/porin Cat.-No. AP00265PU-N with 3T3 cell lysate

## Antibody tools for the detection of loading controls

Acris Antibodies offers a wide range of antibodies to proteins which are suitable for use as loading controls. The table below shows a selection of our recommended antibodies.

### Selected Acris Antibodies panel to loading controls

Name	Property	Host/Isotype	Clone	Reactivity	Application	Format	Catalog-No.
<b>Actin beta</b>							
Actin beta / ACTB	N-term	Mouse IgG	B11V08	Bov, Chk, Hst, Hu, Ms, Por, Rb, Rt, Sh	IF, P, WB	Purified	AM00194PU-N
		Mouse IgG1	G043	All Species	IF, WB	Aff-Purified	AM20709PU-N*
	C-term	Rabbit	-	Hu, Ms, Rt	E, IF, WB	Aff-Purified	AP30136PU-N
		Rabbit	-	Bov, Hu, Ms, Rt	WB	Purified	AP21589PU-N*
		Rabbit	-	Hu, Ms, Rb	WB	Aff-Purified	AP00310PU-N
		Rabbit	-	Hu	E, P, WB	Aff-Purified	AP09293PU-N
359-368	Rabbit	-	Bov, Can, Chk, Fe, GP, Hu, Ms, Por, Rb, Rt, Sh	P, WB	Purified	AP15333PU-S*	
<b>GAPDH</b>							
GAPDH		Mouse IgG1	6C5	Can, Fe, Fish, Hu, Ms, Por, Rb, Rt	E, IF, WB	Purified	ACR001P
		Mouse IgG1	1D4	Bov, Chk, Hu, Mam, Ms, Por, Rb, Rt	IF, WB	Purified	SM7116P*
		Mouse IgG2b	AT8G4	Hu	E, IF, WB	Purified	AM09367PU-N*
		Mouse IgG1	3B3	Hu	E, IF, WB	Aff-Purified	AM20781PU-N*
		Rabbit	-	Hu, Broad	E, IF, P, WB	Aff-Purified	AP09372PU-N
		Rabbit	-	Dros, Fe, Hu, Mky, Ms, Por, Rt	WB	Purified	AP21839PU-N*
<b>Tubulin alpha</b>							
TUBA1B		Mouse IgM	TU-16	All Species	C, E, IF, IP, P, WB	Purified	SM3001P
		Mouse IgG1	TU-01	All Species	C, E, IF, IP, WB	Purified	BM753*
	427-441	Rabbit	-	Av, Hu, Mam	E, WB	Aff-Purified	AP09289PU-N
<b>Tubulin beta</b>							
TUBB / TUBB5		Mouse IgG1	10B1	Hu, Mky, Ms, Rt	E, IF, WB	Aff-Purified	AM20777PU-N*
		Mouse IgG1	A10	Dic, Hu, Ms, Sac, Tet	IF, WB	Aff-Purified	AM20708PU-N*
		Mouse IgM	TU-06	All Species	IF, P, WB	Purified	BM169S
		Rabbit	-	All species	IP, P, WB	Purified	AP15841PU-N*
		Rabbit	-	Bov, Can, Chk, Hu, Mky, Ms, Rt, Xen	P, WB	Purified	AP21644PU-N*
TUBB3 / TUBB4		Mouse IgG1	TU-20	Broad	C, E, F, IF, P, WB	Purified	BM170S*
<b>VDAC/porin</b>							
VDAC	C-term	Rabbit	-	Bov, Hu, Ms, Por, Rb, Rt	WB	Aff-Purified	AP00265PU-N
	185-197	Rabbit	-	Hu, Rt	IP, WB	Aff-Purified	SP5361P
	185-197	Rabbit	-	Av, Hu, Mam, Reptiles	E, WB	Aff-Purified	AP09290PU-N

**Reactivity:** Av: Aves, Bov: Bovine, Can: Canine, Dic: Dictyostelium, Dros: Drosophila, Hst: Hamster, Hu: Human, Ms: Mouse, Por: Pig, Rb: Rabbit, Rt: Rat, Sac: Saccharomyces, Sh: Sheep, Tet: Tetrahymena, Xen: Xenopus

**Application:** C: Immunohistochemistry on frozen sections, E: ELISA, F: Flow cytometry, IF: Immunofluorescence, IP: Immunoprecipitation, P: Immunohistochemistry on formalin-fixed, paraffin-embedded tissue sections, WB: Western blot

\* Various package sizes are available