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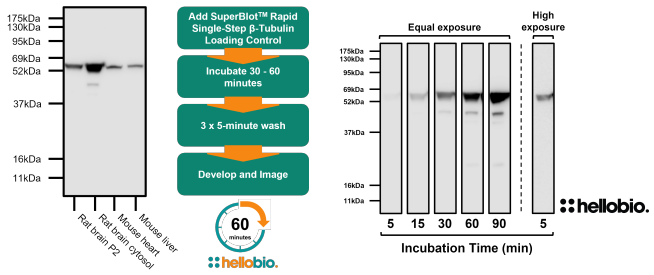
DATASHEET

SuperBlot™ Rapid Single-Step β-Tubulin Loading Control

Product overview

Name	SuperBlot™ Rapid Single-Step β-Tubulin Loading Control
Cat No	HB15002
Biological description	SuperBlot™ Rapid Single-Step β-Tubulin Loading Control is a novel single-step loading control solution for Western Blot that enables the rapid blocking and staining of membranes for β-Tubulin in a single stage: • Simultaneous blocking, primary and secondary antibody incubation • Works in as little as 5 minutes. • Saves hours compared to conventional methods. • Acts as a signal enhancer to increase sensitivity. • Animal product free (recombinant antibodies).
Host	Mouse
Target	β-tubulin
Primary Antibody	Mouse Monoclonal Anti-β-Tubulin (HB6491)
Secondary Antibody	Goat Monoclonal anti-Mouse IgG (H+L) HRP
Description	Rapid, single step β-Tubulin loading control for Western Blotting - get bands in as little as 30 minutes. Part of the SuperBlot™ range of innovative Western Blotting Tools.

Validation data



Product information

Kit contents

This kit contains two components that need recombining before use to create the active reagent:

- Lyophilised premixed Mouse Monoclonal Anti-β-Tubulin (HB6491) and Goat Monoclonal anti-Mouse IgG (H+L) HRP
- 100ml SuperBlot™ Rapid Single-Step Blocking Solution (equivalent to HB9246)

To combine the reagents to create the SuperBlotTM Rapid Single-Step Loading Control:

1. Add around 1ml of SuperBlot™ Rapid Single-Step Blocking Solution with Preservative to the SuperBlot™ Rapid Single-Step Loading Control - Antibody cocktail.
2. Vortex thoroughly and then briefly centrifuge to ensure all liquid is at the bottom of the vial.
3. Transfer the contents of the vial into the remaining SuperBlot™ Rapid Single-Step Blocking Solution with
   Preservative and mix thoroughly.

Please note: Store the combined reagent at 4°C for up to 3 months. If desired, smaller amounts of the

	final buffer can be made up at a time and the individual components snap frozen and stored at -20°C.
Immunogen	Primary: Tubulin preparation from pig brain
	Secondary: Recombinant full length mouse IgG
Clone number	Primary: 1B12
Isotype	IgG
Purification	Primary: Protein G affinity chromatography
	Secondary: Gravity column chromatography
Predicted species reactivity	Human, Mouse, Rat
Tested species reactivity	Mouse, Rat, Human

Tested applications

Applications	WB
Application notes	

Protocol PDF

1. Run SDS-PAGE gel and transfer to PVDF membrane following standard protocols (please see our Western Blot protocol)
2. Following transfer briefly rinse the membrane with PBST or TBST to remove excess transfer buffer then add around 10-20ml of SuperBlot™ Rapid Single-Step β-Tubulin Loading Control.
3. Incubate for 60 minutes for optimal results. Results are available in as little as 15 minutes but 60 minutes provides an optimal balance between speed and signal/noise.
4. Pour off SuperBlot™ Rapid Single-Step β-Tubulin Loading Control (this can be stored at 4°C and reused multiple times).
5. Wash blot 3 times briefly then 3 x 5 minutes with PBST or TBST.
6. Develop with an ECL substrate such as SuperBlot ECL Western Blotting Substrate Kit (High sensitivity).
7. Image the blot using an appropriate imaging system for chemiluminescence.

Positive control	β-tubulin is expressed ubiquitously across nearly all mammalian cell and tissue types. It is also widely expressed in common cell lines (e.g. HEK293, SH-SY5Y, HeLa)
Negative control	β-tubulin is a cytoskeletal enzyme, so complete subcellular fractionation should be sufficient to provide a negative control. Due to its high expression, care should be taken to ensure that fractionation is complete without any cytoskeletal contamination.
Open data link	Please follow this link to OSF

Target information

Other names	Tubulin beta chain, Tubulin beta-5 chain, TUBB
UniProt ID	P07437
Gene name	TUBB
NCBI full gene name	tubulin beta class I
Entrez gene ID	203068
Amino acids	444 (49.7kDa)
Isoforms	β-tubulin has no isoforms other than the main sequence.
Expression	Expressed widely across all cell and tissue types including common cell lines.
Subcellular expression	Expressed in the cytoskeleton as a microtubule component.
Processing	None
Post translational modifications	β-tubulin has phosphorylation sites on multiple residues alongside numerous gamma-glutamylation sites.
Homology (compared to human)	Mouse and rat β-tubulin have a 98.4% and 93.2% identity to human β-tubulin as measured in a BLAST search
Similar proteins	No proteins (other than β-tubulin family members) show significant homology in a BLAST search

Storage & Handling

Storage instructions	Buffer: RT; Antibodies: -20°C, 4°C when combined
Shipping conditions	Items are shipped at room temperature, transfer the antibody cocktail to -20°C upon delivery.
Important	This product is for RESEARCH USE ONLY and is not intended for therapeutic or diagnostic use. Not for human or veterinary use

References

beta-tubulin is a more suitable internal control than beta-actin in western blot analysis of spinal cord tissues after traumatic injury.

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Tubulin: Structure, Functions and Roles in Disease.

Binarová P et al (2019) Cells 8
PubMedID 31652491

The structured core of human β tubulin confers isotype-specific polymerization properties.

Pamula MC et al (2016) The Journal of cell biology 213
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