

Loading Controls for Western Blotting

Your Quick Guide to Loading Control Antibodies

Challenges in Western Blotting

Although Western blotting is one of the most common immunodetection techniques, there are still some critical technical concerns that may be overlooked such as:

- Do isolation or separation protocols affect the integrity of the protein or its post-translational modification?
- Can one differentiate between degradations or aggregations of protein and the relevant products of biological processes?
- When the result is multiple bands, how can one determine which are result, variation, or artifact?

How Loading Controls Address These Challenges

Loading controls can help overcome Western blotting challenges. When identification and measurement of total protein levels across multiple samples is needed, loading controls can be used as internal standards.

Loading controls can:

- Permit quantitation of sample loading across all wells
- Show protein loading variation and may account for observed variations in the target
- Ensure appropriate protein quantitation despite subtle differences in loading amounts

What makes an antibody suitable to be used as loading control?

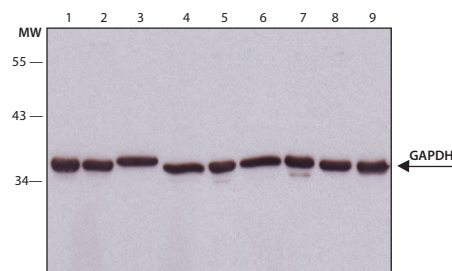
The features of antibodies used for loading controls include:

- Ubiquitously expressed "housekeeping" gene products
- Highly conserved
- High expression level
- Stable under most experimental conditions
- Relatively constitutive expression in most model systems

Considerations for Choosing Loading Control Antibodies

When determining which antibodies to use for your loading control, you should:

- Use internal loading controls that are stably expressed and are minimally affected by experimental conditions
- Select a loading control antibody against a protein known to be constitutively expressed in your sample
- Choose a loading control antibody that covers a wide range of molecular weights (MW), so that it is in a similar MW range, but not the same MW as the target protein

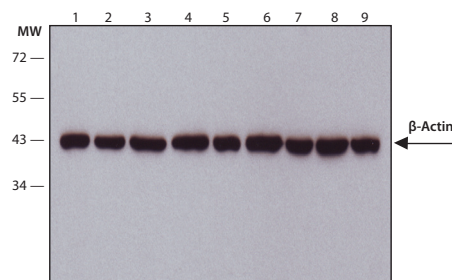


Consistent Expression Across Species

Immunoblotting

Monoclonal Anti-GAPDH Clone: GAPDH-71.1 (Cat. No. G8795).

1. HeLa, 2. JURKAT, 3. COS7, 4. NIH-3T3, 5. PC-12, 6. RAT2, 7. CHO, 8. MDBK, 9. MDCK



Highly Conserved and High Expression Level

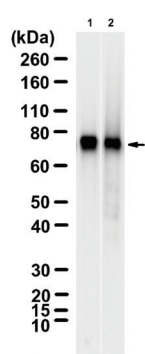
Immunoblotting

Monoclonal Anti-β-Actin, Clone: AC-15 (Cat. No. A5441).

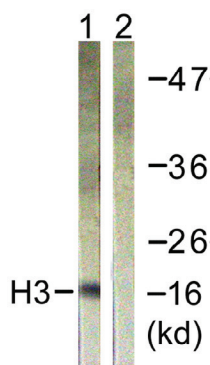
1. HeLa, 2. JURKAT, 3. COS7, 4. NIH-3T3, 5. PC-12, 6. RAT2, 7. CHO, 8. MDBK, 9. MDCK.



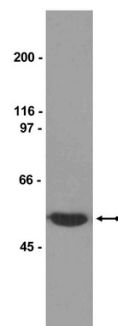
Example covering a wide range of molecular weights



Anti-Transferrin, Cat. No. ZRB1225
Molecular weight - 77k Da



Anti-Histone H3, Cat. No. SAB4500355
Molecular weight -15 kDa

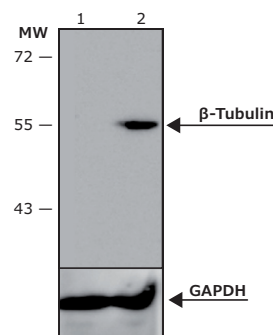


Anti-β Tubulin, Cat. No. 05-661
Molecular weight -50 kDa

Loading Control Example

Anti-GAPDH antibody, (Cat. No. G8795) was used as loading control. HeLa TUBB3 KO (knocked out) Cell Lysate vs. HeLa wild type Cell Lysate were separated on SDS-PAGE probed with Anti-β-Tubulin III antibody produced in Rabbit (Cat. No. T2200).

No antibody staining can be indicated in TUBB3 KO (knocked out) lysate (lane 1). Loading control exhibits the same reactivity vs. two cell lysates demonstrating the product as an ideal loading control.



Antibody Control Options

Cat. No.	Product Description	Species Reactivity	Applications	Size
A5441	Anti-β-Actin antibody, Clone AC-15	Multiple mammalian species	ELISA, WB, IHC, IF	100, 200, 500 uL
A2066	Anti-Actin antibody, Rabbit polyclonal	Human, other vertebrates	IF, IHC, WB	100, 200 uL
05-661	Anti-β-Tubulin Antibody, clone AA2	Human, Rat, Mouse, Bovine	WB	200 ug
CB1001	Anti-GAPDH, Clone 6C5	Multiple mammalian species	ICC, WB	500 ug
G8795	Anti-GAPDH antibody, Clone GAPDH-71.1	Multiple mammalian and avian species	ELISA, ICC, WB	100, 200 uL
G9545	Anti-GAPDH antibody, Rabbit polyclonal	Human, Mouse, Rat	IF, IP, WB	100, 200 uL
AB10527	Anti-VDAC Antibody, Rabbit polyclonal	Multiple mammalian species	WB	100 ug
AB10526	Anti-COX4 (Cytochrome c Oxidase), Rabbit polyclonal	Human	IHC, WB	100 ug
PLA0269	Anti-HSP60 Antibody, Rabbit polyclonal	Multiple mammalian species	IF, IHC, IP, WB	100 uL
07-371	Anti-Histone H2B, Rabbit polyclonal	Human, Chicken	WB	200 ug
05-1352	Anti-Histone H2B, Clone 5HH2-2A8	Human, Mouse, Rat	ICC, WB	100 uL
MABE288	Anti-PCNA, Clone PC10	Human	IF, IHC, WB	200 ug

For Research Use Only. Not For Use In Diagnostic Procedures.

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