

What you need to know about loading control antibodies

Loading control antibodies are crucial for assessing western blotting efficiencies and for equal loading of protein into each well across a gel. These controls help determine whether expression-level differences from sample to sample are due to actual differences in protein levels in a given lysate or from loading variances.

Your checklist for choosing the right loading control antibodies:

Molecular weight

Choose a loading control that has a distinctly different molecular weight than your protein of interest to ensure that the two proteins do not overlap during detection.

Sample type

Based on the sample you are studying, choose the loading control that exhibits high-level, constitutive expression. Loading controls may vary depending on sample type.

Host

In case you want to run a multiplex western blot (probe with primary antibodies for both the protein of interest and the loading control), pick a loading control that has a different host than that of the primary antibody used for your protein of interest.

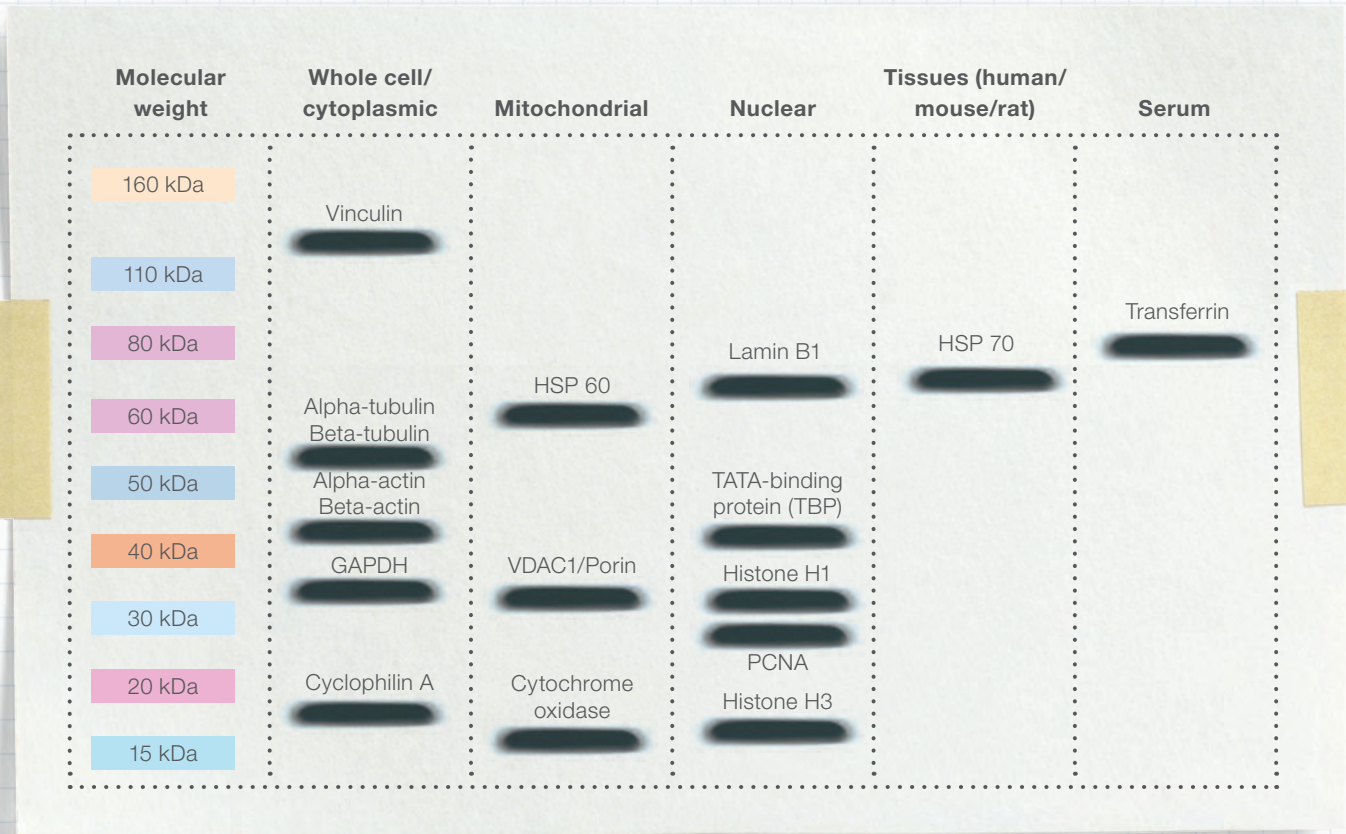
Protein PTMs

When studying protein post-translational modifications (PTMs), the unmodified or total form of the protein of interest will serve as a great loading control, rather than using a generic loading control.

Multiple species coverage

When studying proteins across species, use loading control antibodies that have appropriate cross-species reactivity.

Choose the right-sized loading control for your sample type



Software tools for normalizing gel loading differences
ImageJ

Reminder: check for conjugated antibodies for fluorescent and chemiluminescent western blotting

Loading control antibodies can also be used as complementary antibody stains in immunofluorescence studies