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## Positive control lysate

A positive control lysate is a lysate from a cell line or tissue sample known to express the protein you are detecting. A positive result from the positive control, even if the samples are negative, will indicate the procedure is optimized and working. It will verify that any negative results are valid.

We recommend you check the antibody datasheet, which will often provide a suggested positive control. Always ensure the tissue or cell line you use is from a tested species. Not all the datasheets will have a suggested suitable control, and we recommend the following in these circumstances:

- Check to see if there are any Abreviews for the antibody. Any tissues, cells or lysates that have been used successfully by these customers can be considered a suitable positive control.
- Try looking at the Swiss-Prot or Omnigene database links on the datasheet. These databases will often have a list of tissues that the protein is expressed in. These can also be considered suitable positive controls.
- Check the GeneCards entry for the protein. This will usually provide you with relative levels of expression in various tissues.
- If you still have difficulty finding a suitable control, we recommend doing a quick literature search on PubMed to see which tissues and cells express the protein of interest.

## Negative control lysate

A lysate from a cell line or tissue sample known not to express the protein you are detecting. This is to check for non-specific binding and false positive results.

## Loading control

Loading controls are antibodies to house-keeping proteins, or proteins that are expressed at equivalent levels in almost all tissues and cells.

Loading controls should be used:

- To check that all lanes in the gel contain the same amount of sample. This gives the researcher confidence that differences in the protein of interest are not due to errors while loading the gel.
- To measure the amount of protein present in samples. To check that there has been even transfer from the gel to the membrane during the western blot procedure.

[View loading controls.](#)

## Endogenous control lysate

We recommend including an endogenous control if you are testing a sample of recombinant protein. This should be an essential part of the experimental plans.

There are inherent difficulties with antibody detection of recombinant proteins that need to be considered. Folding of the recombinant protein may be different from the endogenous native form, and may prevent access of the antibody to the epitope. This is particularly the case with tagged proteins.

Always ensure tags are placed on the N or C terminal end of the recombinant protein. Most importantly, always ensure the recombinant protein includes the immunogen sequence for the antibody you are using. An endogenous positive control is important to validate the results, as well as to indicate how well the reagents (e.g. antibodies) and procedure are working.

## No primary antibody control

This is when the primary antibody is not added to one strip of membrane. Secondary antibody only is added. This indicates if any non-specific binding or false positives may be due to non-specific binding of the secondary antibody.

Antibody dilution buffer containing no antibody is used in place of the primary antibody solution at this point in the procedure. The secondary antibody is incubated on the same sample in the same way as usual.