

Tips and tricks of the trade

Second-Hand Antibodies

Many researchers are afraid that Western blots don't work properly with 'recycled' antibodies and, therefore, routinely use fresh antibodies for every blot. A recent study however shows, that these concerns are arbitrary.

Lab Hint

Western blotting is the most common method for detection and quantification of specific proteins. The costs of this method are mainly determined by the costs of the antibodies needed for detection. This logically only applies to labs that depend on commercial antibodies. So, if your lab produces large amounts of antibodies you might just skip this article. For all others, reading on may help to cut the lab's antibody expenses.

How to cut costs for antibodies for Western blotting is, in fact, no secret to most of the scientific community. The simple answer is to use the antibody solution on one blot and re-use it on the next one. But the answers to the questions how often antibodies can be reliably re-used and how they should be stored in the meantime are usually not so clear. These answers are important because you probably do not want to repeat time-consuming Western blotting, especially if the samples loaded are precious to you.

Antibody re-use sceptics

Although the possibility of re-using antibodies is frequently discussed in many internet forums, it has not been addressed up to now in a scientific manner. The recently published article "Analysis of the effectiveness of re-used primary and secondary antibodies in Western blotting analysis" (K. Boonrod *et al.*, *Anal. Biochem.* 2010 Feb 1; 397 (1):124-5) has addressed exactly this – and the answer is quite simple, "Yes you can! (re-use your antibodies)". The results in the article are anything but surprising and, hopefully, will remove any remaining objections by "antibody re-use sceptics".

In the paper mentioned above, the re-usability of eight different antibodies from four different suppliers was systematically

tested. Five primary tag-specific antibodies (anti-HA-peroxidase, anti-GST-peroxidase, anti-MBP, anti-GFP, anti-His) and three secondary antibodies (anti-rabbit-peroxidase and two anti-mouse-peroxidase) were re-used to detect their respective targets; the storage conditions at 4 °C and -20 °C were also compared.

To make it short, six of the tested antibodies could be re-used four times without any problems and the other two worked perfectly for two and three repetitions. The different storage conditions (4 °C and -20 °C) did not affect the antibody's performance, regardless of the suppliers' recommended storage conditions. The fact that the test antibodies came from different suppliers and had strongly diverse specificities indicates that these findings can be transferred to most available antibodies. For storage of antibodies at 4 °C, microbial growth can be a problem as antibody diluents often contain skimmed milk or BSA. Sodium azide, an antimicrobial agent, can therefore be added to prevent this. However, it should be avoided when storing peroxidase-coupled antibodies, since sodium azide interferes with the detected signal. Another problem when re-using antibodies is the loss of the antibody solution due to multiple transfers. This loss



Labs performing Western blots in 'mass production' may save a lot of money when re-using antibodies.

can be limited by storing the antibody solution in the container used for the blot incubation.

No mysterious trick

At least by now, the issue of re-using antibodies should no longer be the mysterious trick, about which nobody is really sure. Instead, it should be a standard procedure applied in every lab that prefers to use its funding in a smart way. So, the next time you start an experimental series with a new antibody, one of the first actions should be the assessment of the re-usability of your antibody. Your group leader will surely be grateful for that and, who knows, maybe he will invest the saved money in other exciting projects, like group outings.

KAJOHN BOONROD AND MARK LAIBLE

Do you have any useful tips?

Contact us at:

editors@lab-times.org