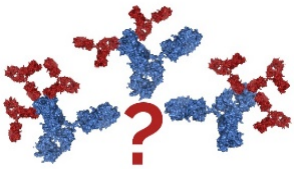
The Antibody Society presents:



Antibody Validation Webinar Series

WEBINAR 5: Really? Out of Control(s)?

Moderator: **Dr. Simon Goodman**, The Antibody Society

Speaker: **Dr. Giovanna Roncador**, Centro Nacional de Investigaciones Oncológicas and EuroMabNet

First Webcast: January 15, 2020

Now available On Demand

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Questions and Answers from the live Webcast on January 15, 2020

Question	Answer
Are there are other methods (alternative to cell transfection) that you can use to check antibody cross reactivity?	The use of transfected cells to check antibody reactivity is the most simple and cheapest method to check antibody cross reactivity. You can also use WB or ELISA using the protein of interest. Also now, there are available protein tissue arrays that contain a large number of protein and will allow you to check antibody cross-reactivity against a large part of the entire human proteome.
Would you please comment on the multiple bands observed in the Western blots in your slides on validation?	Yes, thank you this is a good question. We have observed a double band only in tumor samples. For this we have performed a proteomic analysis confirming that the second band is due to protein phosphorylation.
How can I prepare a small tissue array for antibody validation?	The conventional construction of a TMA block involves the use of a commercial TMA machine that can be quite expensive. It is now possible to use a mechanical pencil (that is commercially available) that allows you to construct a tissue array block in minutes, simply by punching the donor tissue cores using punch needles with plunger and inserting into the pre-made paraffin recipient block without the need of specialized equipment.
Do you always use an unspecific isotype control antibody for your immunostaining?	**GR**: We do not use unspecific isotype control. What we do is omit the primary antibody to confirm that the secondary antibody is not giving unspecific staining. **SG**: A variant may be to use a specific isotype matched antibody, with a well validated staining pattern expected to be different from the antibody under test (ie. a membrane target when the test antibody is expected to be a cytoplasmic target)."
Why should we use more than one Ab against the target?	Testing several different antibodies in parallel and observing any common patterns of antibody reactivity can significantly strengthen confidence in the validation data generated. This is particularly important when unexpected or novel findings are observed. We always validate our antibodies with at least other two Abs against the same target (if available).
Thanks for the answer. However, is it possible that, by omitting a primary control Ab, you could misinterpret the binding of your Ab via the Fc domain to an FcRn receptor?	Yes, you are right. This is a good question, and it can be a problem if your antibody is against plasma cells. You could use an Fab2 fragment, of course.

When is the next episode?	**SG response**: Episode #6 will be broadcast January 22, 2020, 10am ET / 16:00 CET. All episodes in this webinar series will be made On Demand after the initial broadcast. Go to **our Learning Center** for the registration links.