

Ten basic rules of antibody validation

– A practical checklist¹

Rule #1*: Definition of the antibody

- ☐ Clone number, antibody ID, or order number is available (for monoclonal antibodies)
- ☐ Order and batch number are available (for polyclonal antibodies)

Rule #2*: Definition of the target

- ☐ Antigen, and/or epitope or hapten structure is given
- ☐ A positive control is defined
- ☐ A calibration curve is given
- ☐ Native or denatured status of the antigen is defined

Rule #3*: Binding selectivity ("Cross-reactivity")

- ☐ Cross-reactivities of structurally similar compounds have been tested
- ☐ Suitable negative controls have been tested

Rule #4*: Concentrations of antibodies and additives

- ☐ The concentration and method of determination of the antibody are given
- ☐ The composition of the product including buffers and additives is given

Rule #5*: Documentation

- ☐ Comprehensive documentation about the product is offered
- ☐ Relevant scientific references are given

Rule #6: Binding strength of antibody/antigen complex

- ☐ Affinity constant or equivalent has been established
- ☐ Valency of the complex was considered

Rule #7: Influence of non-target substances ("Matrix-effect")

- ☐ Matrix tests have been performed

Rule #8: Stabilization and storage

- ☐ Advice for stabilization and storage is given

Rule #9: Application protocols

- ☐ Relevant application protocols are available
- ☐ Species and isotype and/or sequence information are given

Rule #10: User feedback ("Open Science")

- ☐ An online platform for user feedback is provided
- ☐ User comments and/or ratings on this specific product are available

Of the five critical rules 1-5 (*), at least one box per rule needs to be checked, otherwise, the antibody is not sufficiently validated on a basic level. Any scientific application is discouraged in this case without additional validation efforts. If other rules are not met, the applicability of the product may be limited. This list is not necessarily exhaustive and can be extended, depending on the envisaged application.

¹ Michael G. Weller, Federal Institute for Materials Research and Testing ([BAM](https://www.bam.de)), Berlin, Germany, Version 1.1, 2018, michael.weller@bam.de