5 Pillar Validation

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We understand how important it is to provide high quality, well validated research reagents. In addition to our industry-leading validation procedures, we will now implement several new methods for antibody validation, in accordance with recommendations instigated by the International Working Group for Antibody Validation (IWGAV)*.

Learn more about our ongoing validation initiatives and reproducibility legacy at RnDSystems.com/Reproducibility

Look for these badges on the 2,000+ validated antibodies available at Novusbio.com/Antibodies & RnDSystems.com/AntibodyList

Genetic Strategy

Expression of the target protein is compared before and after gene knockout or knockdown using CRISPR/CAS9 or siRNA/shRNA.

WT

KO
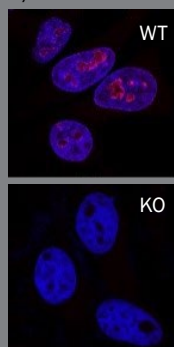
Results showing substantially reduced protein expression following knockout or knockdown demonstrate the antibody's unique specificity for the target protein.

Orthogonal

An antibody-dependent strategy is compared against an antibody independent strategy. A correlation between the results from the two strategies confirms accurate specificity.
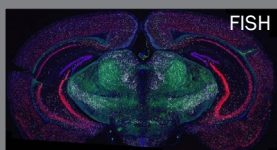
FISH

Antibody independent validation techniques include in situ hybridization, fluorescent in situ hybridization (FISH), quantitative PCR, RNA-seq, and mass spectrometry.

Independent

The data generated using several antibodies (ideally targeting different epitopes on the same protein) are compared (e.g. molecular weight and cellular localization).
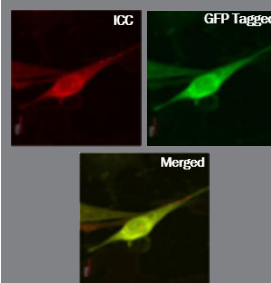
IP/WB

kDa
460–

268–
238–

171–

117– ← HIF1-Alpha

71–

55–
41–

+	–	–	Ab₁
–	+	–	Ab₂
–	–	+	Control Ab

IP
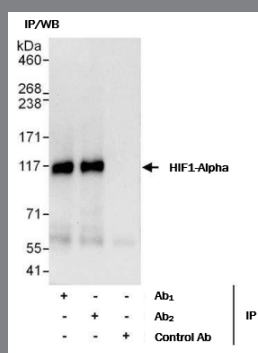
Consistent results across all tested antibodies imply selectivity to the target protein.

Expression of Tagged Proteins

A tagged protein is used as a standard for comparison against an untagged protein in western blotting or immunocytochemistry.
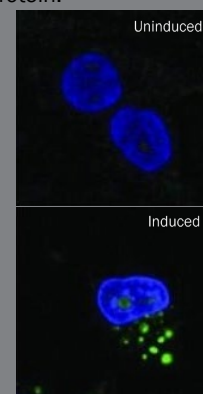
ICC   GFP Tagged

Merged

For example, in ICC if the distribution of the tagged protein overlaps with the immunofluorescence signal then the antibody is specific.

Biological Strategies

These strategies use defined biological or chemical modulation of protein expression to demonstrate antibody specificity to the target protein.

Uninduced

Induced

The data is compared across multiple cell lines (including positive and negative expressing cells) and multiple species, if applicable.

*Uhlen M, Bandrowski A, Carr S, Edwards A, Ellenberg J, et al. (2016) A proposal for validation of antibodies. Nature Methods 13(10):823-7

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