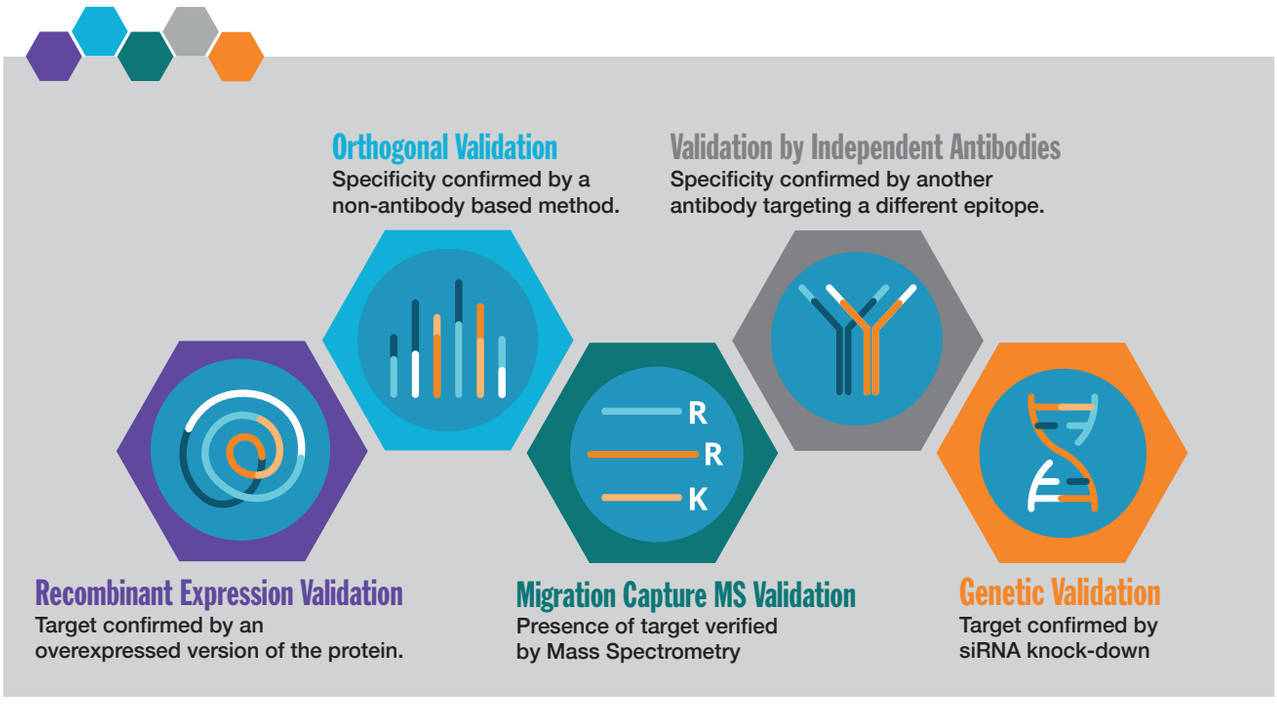
Enhanced Validation

ENHANCED VALIDATION



Enhanced Validation

The extra layer of security in antibody validation

ATLAS ANTIBODIES

Antibody Validation

Validation of antibodies is an experimental confirmation that an antibody is suitable for its intended use. It is important to understand that antibody validation is application- and context specific. Atlas Antibodies provides highly characterized antibodies further strengthened by enhanced validation, as recommended by leading researchers and described in this paper.

Triple A Polyclonals™

Triple A Polyclonals are originally developed within the academic Human Protein Atlas (HPA) project (proteinatlas.org). HPA uses the antibodies to characterize all human proteins in the majority of normal and cancerous tissues, as well as on a subcellular level. The uniqueness and specificity of the antibodies used, originate from a thorough selection of antigen regions, affinity purification of the polyclonal antibodies, a stringent selection of approved antibodies and validation using different methods.

PrecisA Monoclonals™

Atlas Antibodies also provides a selected number of mouse monoclonal antibodies, under the brand name PrecisA Monoclonals. PrecisA Monoclonals are developed with careful antigen design and are isotyped for multiplex possibilities.

Transparency of Data

- All antibody characterization data achieved using different applications are freely accessible on the Human Protein Atlas portal.
- For immunohistochemistry (IHC), every antibody is supplied with 500 images, from 44 normal and 20 cancer tissues.
- The antigen sequences are provided on the product pages for all antibodies.
- Precise epitope information is supplied for PrecisA Monoclonals (where available).
- Our scientific support team will be happy to share anything you may like to know about production and quality control of each antibody.

Enhanced Validation of Antibodies

To further strenghten the reliability of our antibodies we apply enhanced validation, based on the recommendations by the International Working Group for Antibody Validation (IWGAV), as published in Nature Methods, Uhlén *et al*[1]. The group consists of researchers from institutions in USA and Canada, such as Stanford University, Yale University, MIT, UCSD, University of Toronto, NIH, EMBL, Niigata University in Japan and the Science for Life laboratory in Sweden.

In the article, five conceptual pillars for antibody validation are suggested to be used in an application specific manner. Based on these pillars, HPA and Atlas Antibodies have developed an Enhanced Validation Strategy, resulting in the following five methods:

Five Conceptual Methods

1. Genetic validation:
Target confirmed by siRNA knock-down.

2. Orthogonal validation:
Specificity confirmed by a non-antibody based method.

3. Independent antibody validation:
Specificity confirmed by another antibody targeting a different epitope of the protein.

4. Recombinant expression validation:
Terget confirmed by an overexpressed version of the protein.

5. Capture MS validation:
Presence of target verified by Mass Spectrometry.

At least one of the methods must be used for an antibody to receive Enhanced Validation Status in a specific application.

Enhanced Validation

1. Genetic Validation

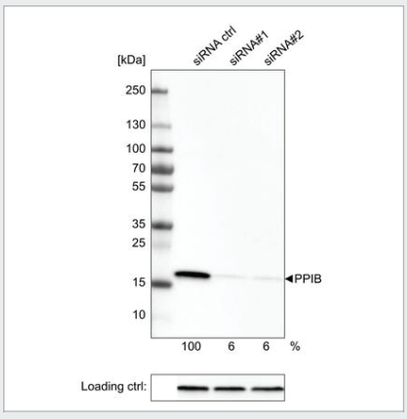


Figure 1.
Example of genetic validation by siRNA knock down in Western blot using the Anti-PPIB antibody. U-251 cells have been transfected with control siRNA and two target specific siRNA probes. Downregulation of antibody signal confirms target specificity. The remaining intensity relative control lane is indicated as a percentage.

2. Orthogonal Validation

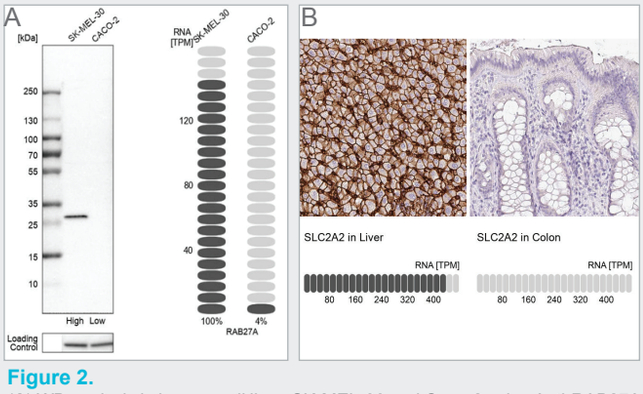


Figure 2.
(**A**) WB analysis in human cell lines SK-MEL-30 and Caco-2 using Anti-RAB27A antibody (HPA001333). Corresponding RAB27A RNA-seq data (TPM values) is presented for the same cell lines. **(B)** IHC staining of liver and colon tissues using the Anti-SLC2A2 antibody (HPA028997). The corresponding RNA-seq data (TPM values) for the same tissues are presented below. In both examples, samples with known high and low RNA expression are chosen and correlation to antibody signal is shown by both WB and IHC.

1. Genetic Validation

The target protein can be down-regulated on a genetic level using siRNA or CRISPR-Cas9. If knock-down (by siRNA) or knock-out (by CRISPR) of the corresponding gene correlates with absence or decrease of antibody signal, the antibody is shown to be specific for its target. This is exemplified in **Figure 1** where the Anti-PPIB antibody AMAb91245 is used in Western Blot (WB) analysis in U-251 cells. The antibody signal is down regulated in the cells silenced by PPIB siRNA.

2. Orthogonal Validation

By using a non-antibody based method for target quantification, the antibody signal can be validated by comparing the results from the different methods across multiple samples.

One approach is to compare the antibody staining intensities over multiple samples with varying expression of the target gene, with RNA-seq data in the same samples. This is illustrated in **Figure 2**, using WB analysis in human cell lines SK-MEL-30 and Caco-2 using Anti-RAB27A antibody HPA001333. Corresponding RAB27A RNA-seq data (TPM values) are presented for the same cell lines.

3. Validation by Independent Antibodies

Two antibodies directed against different regions of the same target protein may validate each other when compared in a set of relevant tissues. This is exemplified in **Figure 3** with two antibodies directed against different regions of the A2ML1 protein used in the immunohistochemistry application. Both antibodies show positive staining in liver, but are negative in tonsil, colon and kidney.

4. Recombinant Expression Validation

An antibody signal can also be validated using an over-expressed or tagged version of the target protein.

When over-expressing the target protein in a cell line, the antibody is validated by comparing the signal from the over-expressed version with the unmodified endogenous target protein. This approach is exemplified in **Figure 4A**.

The target protein may also be tagged by an affinity tag or a fluorescent protein. The pattern displayed by the tagged target protein is matched to the antibody signal.

A match confirms that the antibody recognizes its target protein. Validation by tagged proteins can be applied in immunocytochemistry-immunofluorescence (ICC-IF) and is shown in **Figure 4B**.

5. Migration Capture MS Validation

In this method, the staining pattern and the protein size detected by the antibody is compared with results obtained by a capture Mass Spectrometry (MS) method. The band generated by the antibody in WB should correlate with the target protein identified by MS in terms of gel migration.

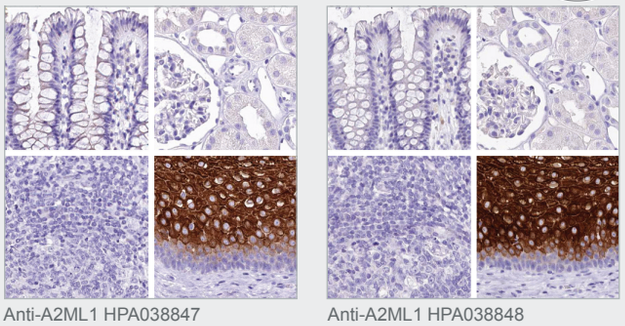
Atlas Antibodies and Validation

At Atlas Antibodies, we take extra care to validate our antibodies in IHC, WB, and ICC-IF.

Our antibodies are extensively validated in collaboration with the Human Protein Atlas project (proteinatlas.org), and implemented by using Enhanced Validation as an extra layer of security.

Enhanced validation offers increased security of antibody specificity in a defined context. This is ensured by using the most relevant validation method for each combination of protein, sample, and application.

3. Validation by Independent Antibodies



Anti-A2ML1 HPA038847 Anti-A2ML1 HPA038848

Figure 3.
Two Anti-A2ML1 antibodies (HPA038847 left and HPA038848 right) targeting different regions of A2ML1 show similar staining patterns in IHC. Antibody stainings across relevant positive and negative tissues are similar between the two, and the antibodies validate each others staining pattern in IHC. Here, the two antibodies are used for staining esophagus (positive for A2ML1 using both antibodies), tonsil, colon and kidney tissues (negative for A2ML1 using both antibodies).

4. Recombinant Expression Validation

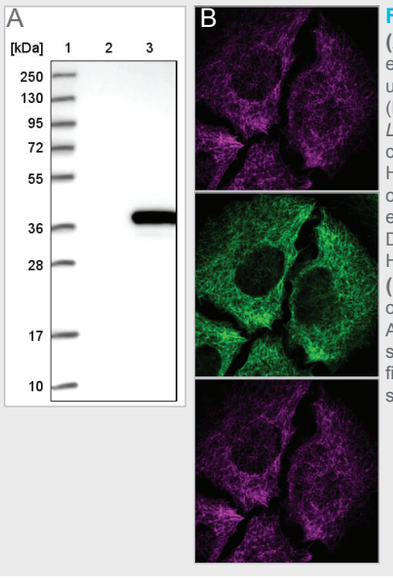


Figure 4.
(A) Example of recombinant expression validation in WB using the Anti-ACY3 antibody (HPA039219).
Lane 1: marker; *Lane 2*: negative control (vector only transfected HEK293T lysate); *Lane 3*: ACY3 over-expression lysate (Co-expressed with a C-terminal myc-DDK tag (~3.1 kDa) in mammalian HEK293T cells, LY408962).
(B) Immunofluorescent staining of transgenic HeLa cells using Anti-NES antibody HPA006286 shows positivity in intermediate filaments (in green). Antibody staining overlaps with GFP

ENHANCED VALIDATION GUIDELINES

Validation Method	Genetic	Independent Antibodies	Orthogonal	Recombinant Expression	Migration Capture MS
Validation Principle	Genetic silencing of target protein	Different antibodies binding a different epitope on the target protein	Compared to non-antibody-based method	Over-expression or tagged protein	Presence of target protein verified by Mass Spectrometry
Suitability	When target is endogenously expressed in cell lines	When 2 antibodies against 2 different epitopes of the target are available	When target is essential or impossible to downregulate	No or low target expression in endogenous samples	When target can be detected in Mass Spectrometry
Limitation	When target is essential or impossible to downregulate	Requires knowledge of antigen sequence	—	Availability of overexpressed lysates. Not for in-vivo use	When target can not be detected in Mass Spectrometry
Application	WB ICC/IF	WB ICC/IF IHC	WB ICC/IF IHC	WB ICC	WB

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