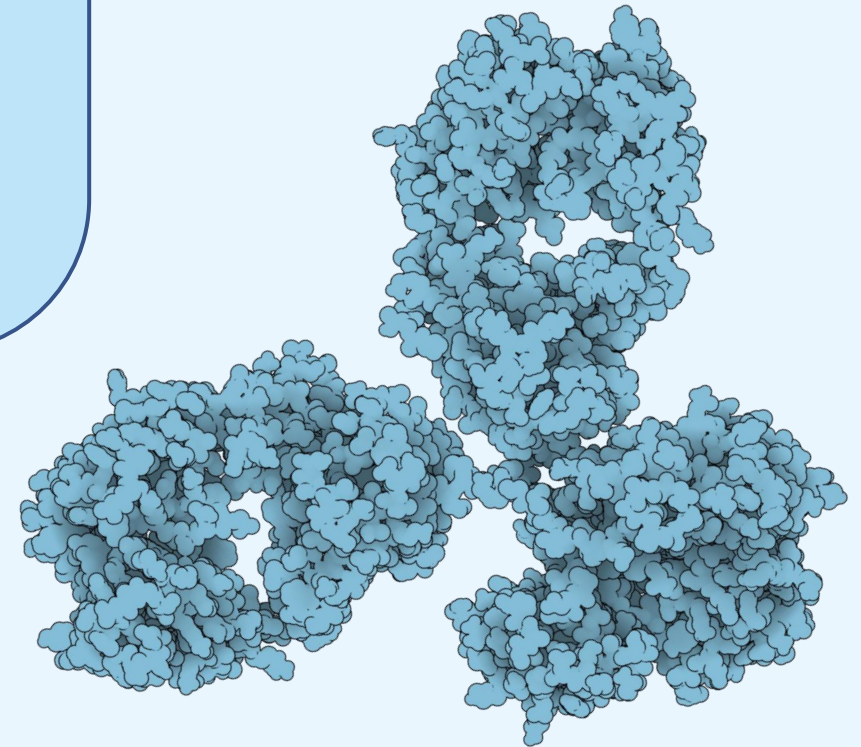


The Antibody Trap: Common Misconceptions That Can Compromise Your Results

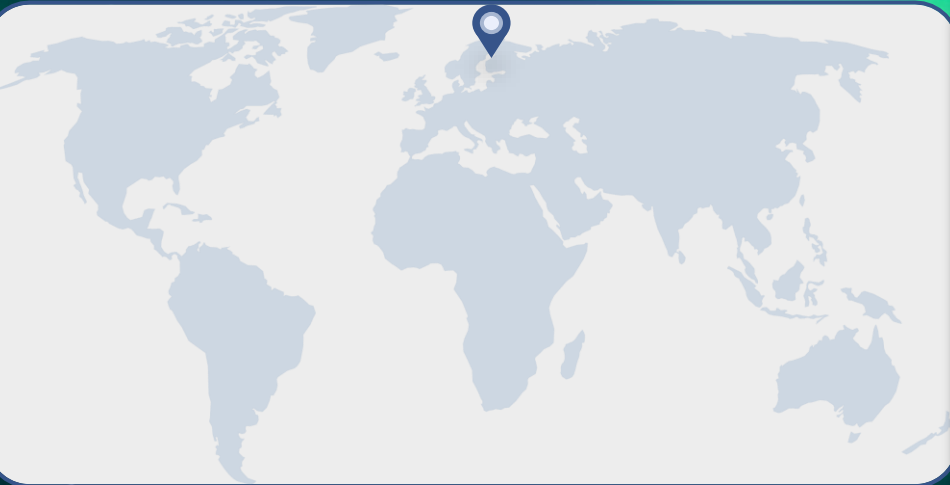
Dr. Joanna Porankiewicz-Asplund, Technical Support Manager at Agrisera, part of Thermo Fisher Scientific
2026-09-24

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About Agrisera

Agrisera
Part of Thermo Fisher Scientific



- Established in 1985
- 85 employees
- Part of Olink since 2020
- Part of Thermo Fisher Scientific since June 2024
- ISO 9001 certified





2025

WINNER

AGRISERA

ANTIBODY SUPPLIER SUCCEEDING
IN PLANT SCIENCE

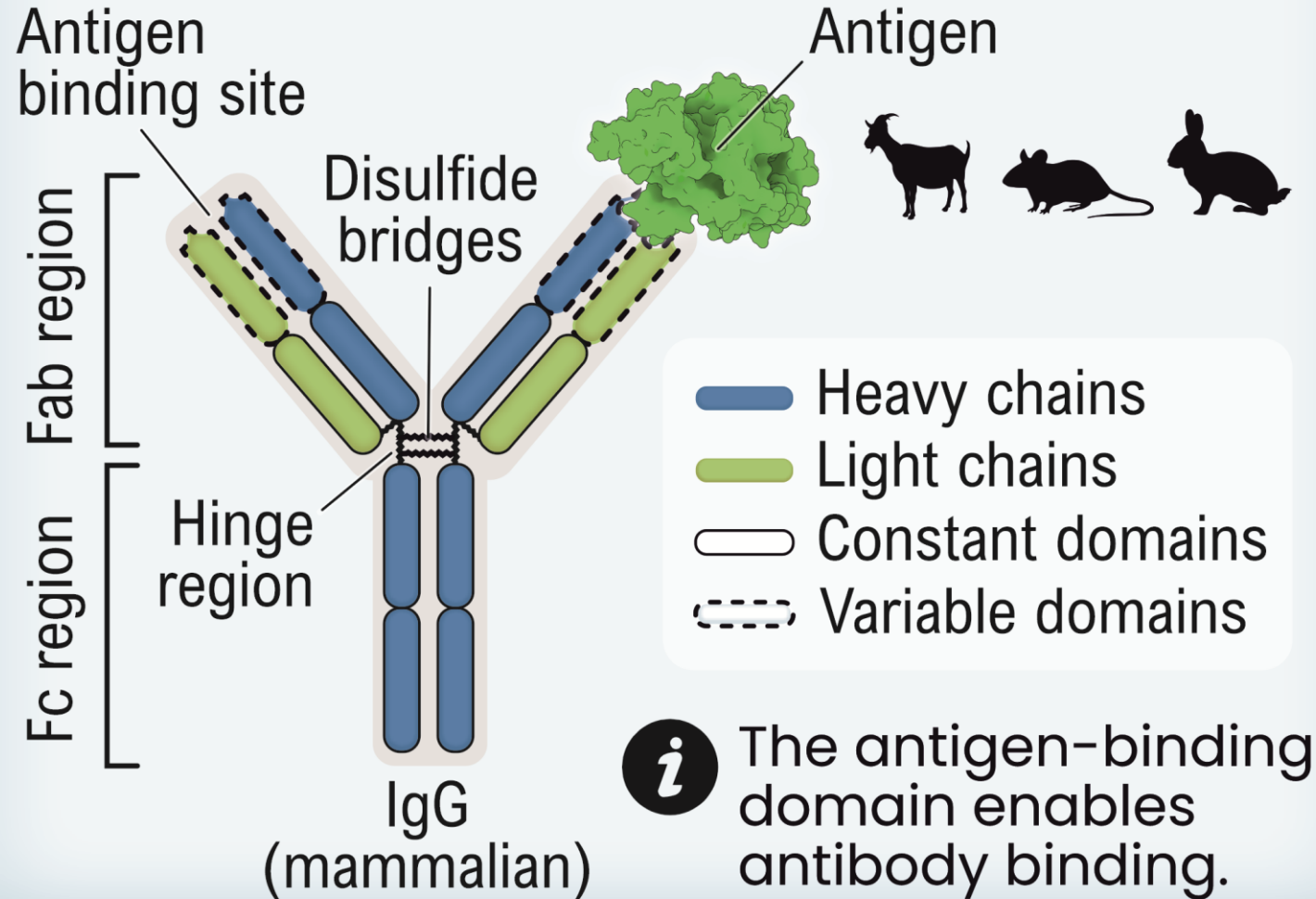


Agrisera Antibodies were cited in over 10 000 publications over the last 25 years.

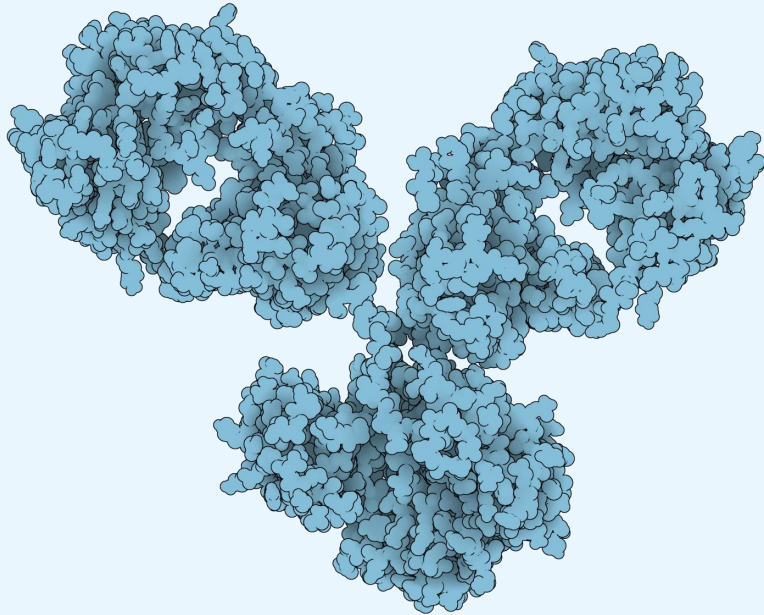
Agrisera primary antibodies are detecting:

- plant, algae and diatom proteins
- hormones
- epitope tag antibodies
- secondary antibodies

Simplified antibody structure

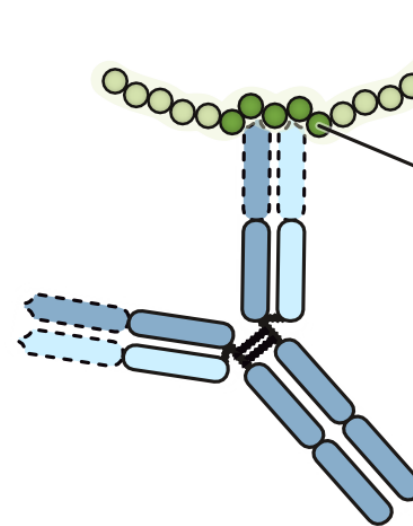


Difference between antigen, immunogen and epitope.



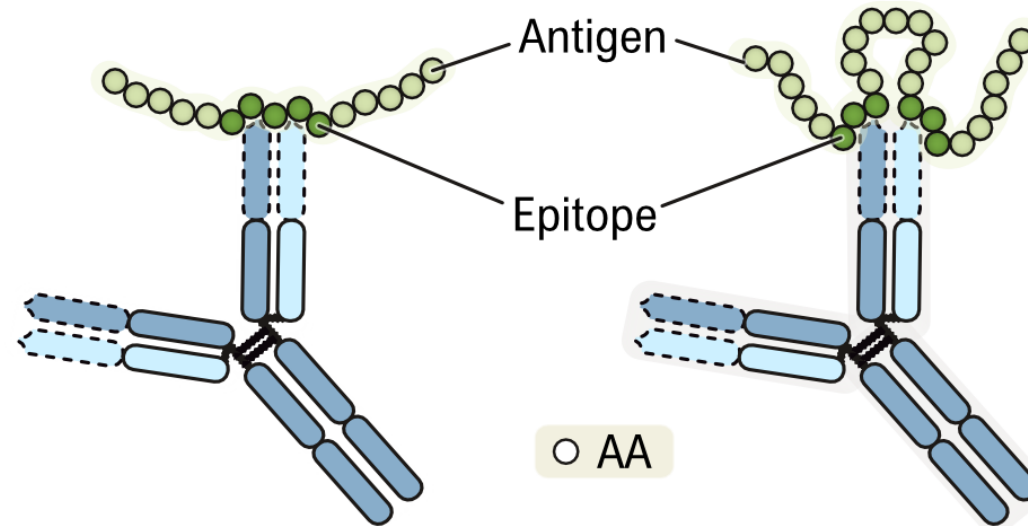
What is an Epitope?

Linear Epitope



i Typically ~5-15 contiguous residues. Often detected in denaturing assays (e.g., WB)

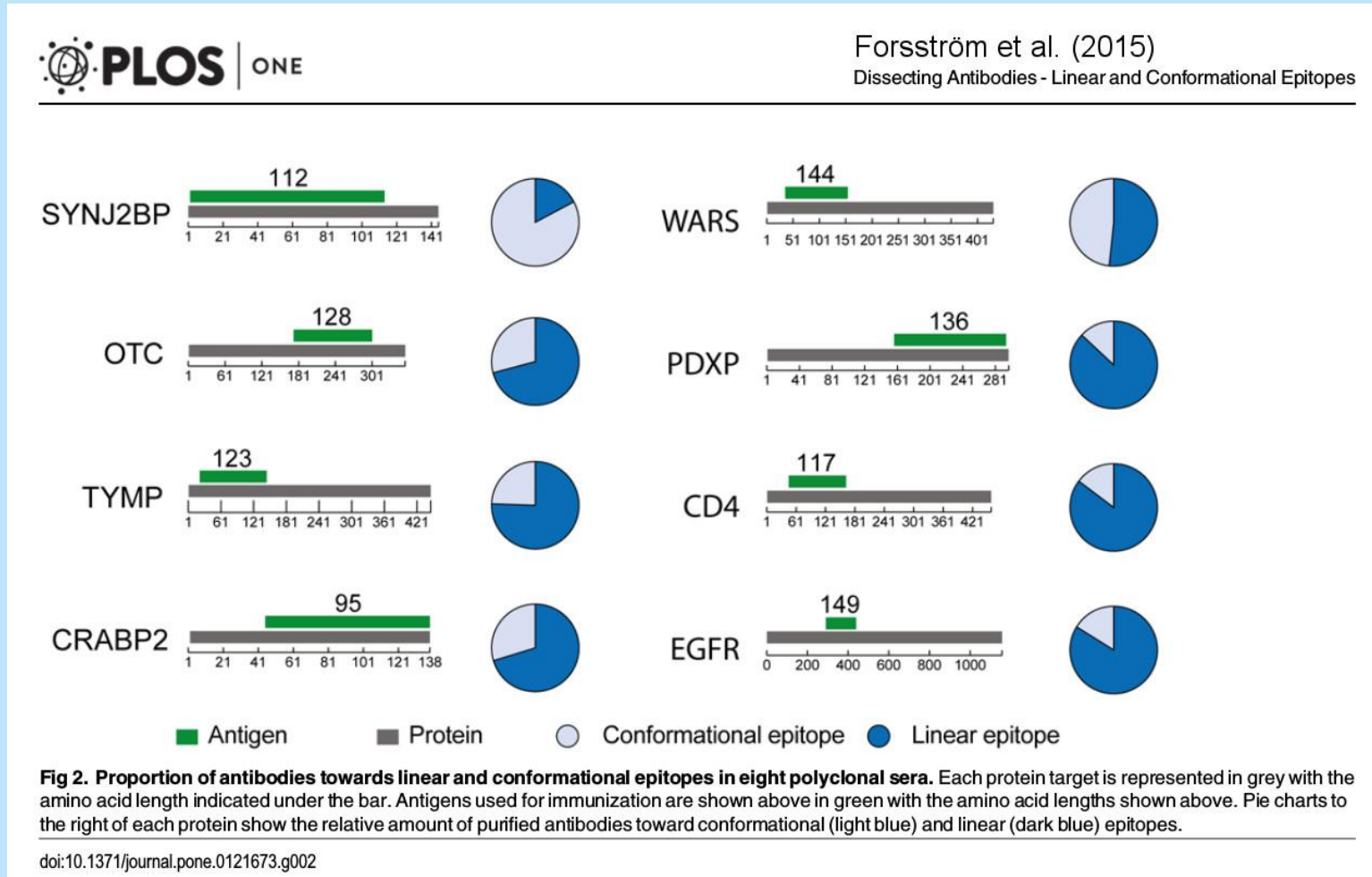
Conformational Discontinuous Epitope



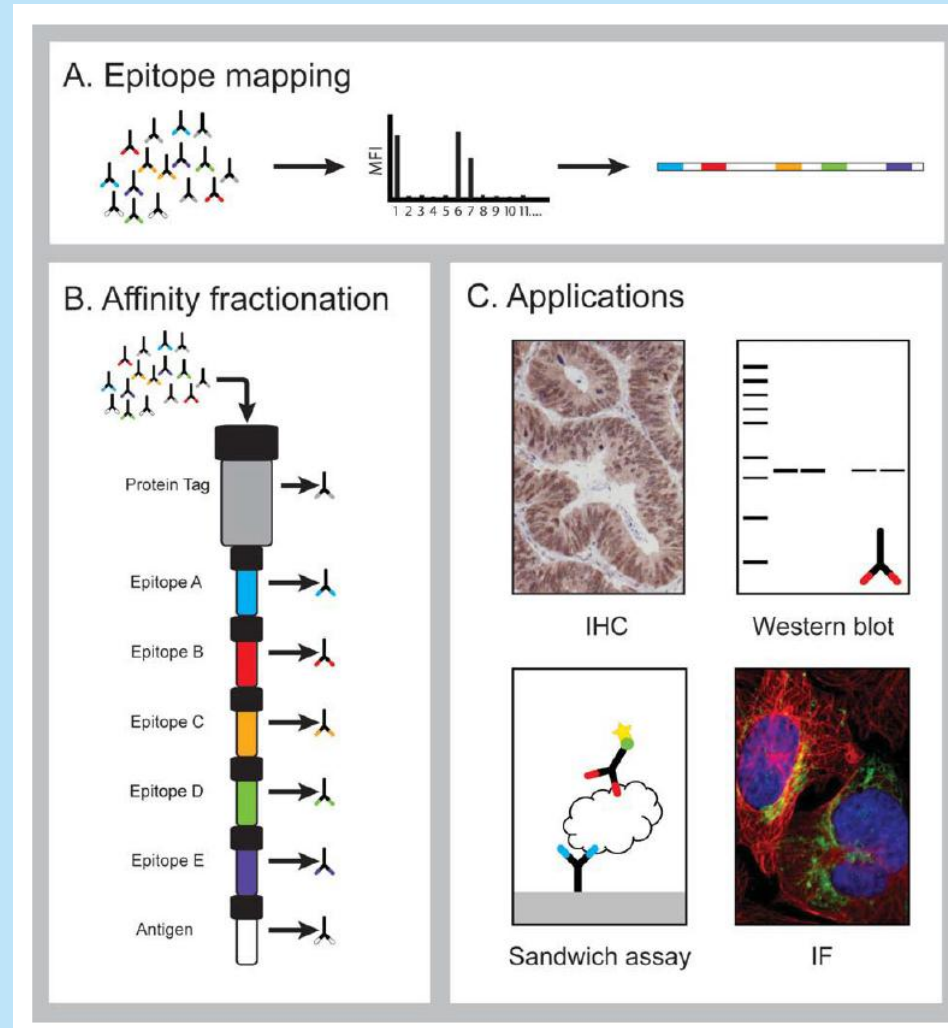
○ AA

i Typically ~10-25 noncontiguous residues total (surface patch; schematic subset shown). Often detected with folded/native antigen (e.g., IP)

Types of epitopes

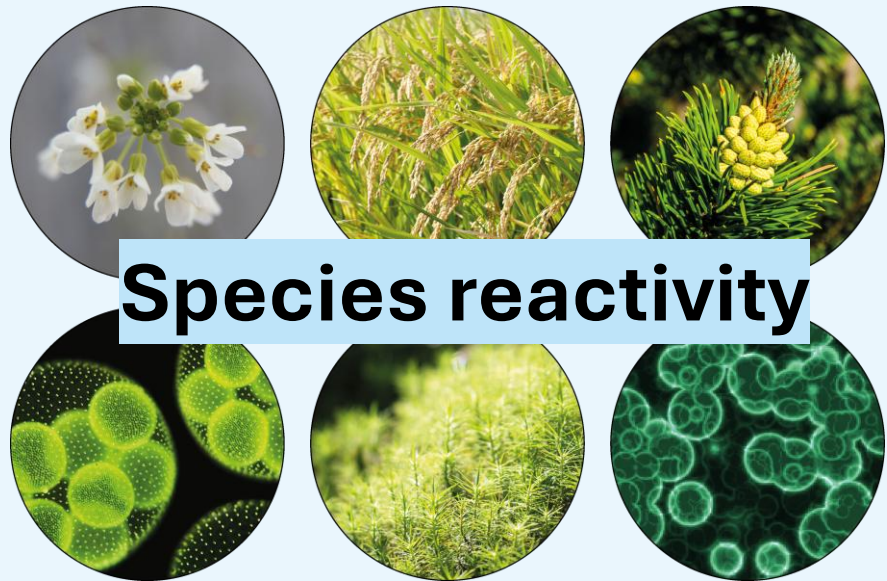


Example of polyclonal antibody epitope recognition

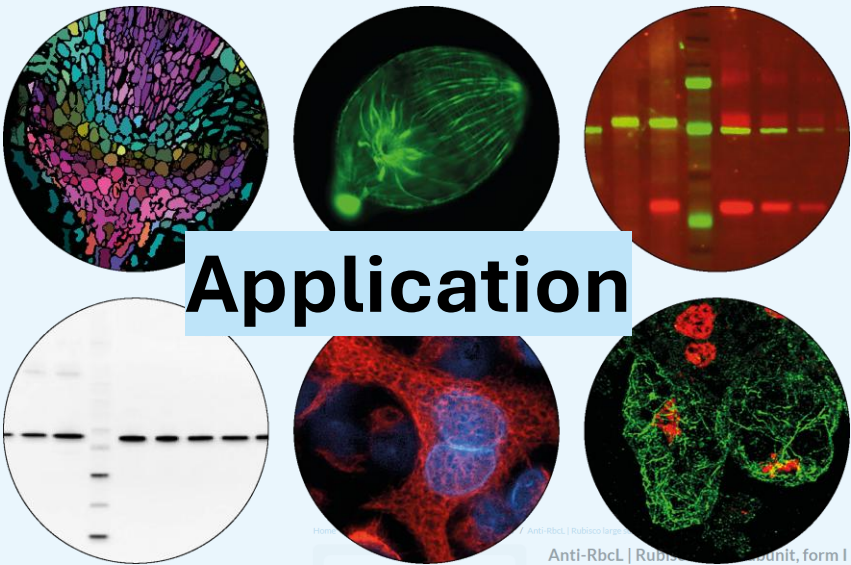


Source: [Generation of monospecific antibodies based on affinity capture of polyclonal Antibodies.](#) Hjelm et al. 2011.

The crucial antibody-selection steps: S-A-C-V



Species reactivity



Application



Clonality

Anti-Rbcl | Rubisco large subunit, form I (rabbit antibody)

AS03 037 | Clonality: Polyclonal | Host: Rabbit | Reactivity: global antibody and compartment marker for higher plants, lichens, algae, cyanobacteria, dinoflagellates, diatoms

Product no: AS03 037

329 € 1 Add to cart

Customer reviews ★★★★★

Delivery: 3-6 business days

DATA SHEET IN PDF

PRODUCT INFO

Immunogen: K111-conjugated synthetic peptide conserved across all known plant, algal and cyanobacterial Rbcl protein sequences (form I L850 and form II L2), including: *Arabidopsis thaliana* P00440, *Hordeum vulgare* P05610, *Oryza sativa* P0C5L0, *Chlamydomonas reinhardtii* P08177, *Synechococcus* PCC 7920 A5C3G5

Host: Rabbit

Clonality: Polyclonal

Purity: Serum

Format: Lyophilized

Quantity: 50 µl

For reconstitution, add 50 µl of sterile water

Store lyophilized/reconstituted at -20°C; once reconstituted make aliquots to avoid repeated freeze-thaw cycles. Please remember to spin the tubes briefly prior to opening them to avoid any losses that might occur from material adhering to the cap or sides of the tube.

Immunofluorescence/confocal Immunolocalization (IL) (IF), Immunogold (IG), Tissue Printing (TP), Western blot (WB native and denatured)

Validation data

**Plant science: > 50 model species in plant science contain
>25 000 proteins/species**

Oryza sativa



Zea mays



Spinacia oleracea



Arabidopsis thaliana



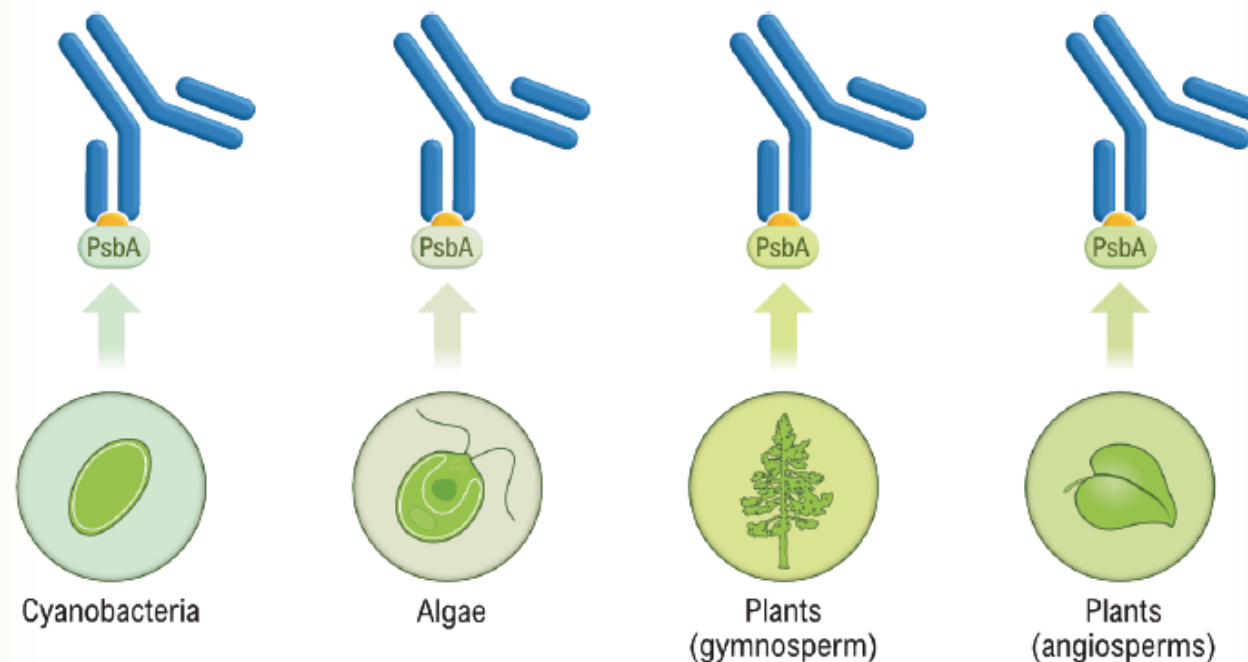
Chlamydomonas reinhardtii



[Free images for download of
model species&pathways](#)

Multispecies reactivity

2. Broad Species Coverage



Outcome: One global antibody can be used for the detection of the target protein in different species (from cyanobacteria to higher plants).

 **1**
Global
Antibody

Reliable detection and
quantification across
1000+ plant species



Global Antibody Collection

FTSIVGNVGFKAL AALRLEDLRIPPAYKTFQGPPhGIQVERDKLNKYGRPLLGTICKP
FTSIVGNVGFKALRALRLEDLRIPPAYKTFVGGPPhGIQVERDKLNKYGRLLGTICKP
TASIIGNVGFKAVSALRLEDMRIPHSYLKTFQGPATGVIVERERLNKYGIPLLGATVKP
..**.....*****..**.******.*.....*****...***..**

GHYLNATAGTCEEMIKRAVFARELGVPIVMHDYLTGGFTANTSLSHYCRDNGLLLIHRA
GHYLNATAGTCEEMMKRAVCAKELGVPIIMHDYLTGGFTANTSLAIYCRDNGLLLIHRA
GSYLNITAGTMEEVYKRAEYAKTVGSIVVMIDLVMG-YTAIQSAAIWARDNDLILHLRA

* * * * *

MHAVIDRQKNHGMHFRVLAKALRLSGGDHIHAGTVVGKLEGDRESTLGFVDLLRDDYVEK
 MHAVIDRQRNHGIHFRVLAKALRMSGGDHLHSGTVVGKLEGEREVTLGFVDLMRDDYVEK
 GNSTYARQKNHGINFRVICKWMRMCGVDHIHAGTVVGKLEGDPLMIKGFYDTLLTHLNV
 .. **.*.*.*.*.*.* * .*. * **.*.*.*.*.*.*.*.*.* ** * ..

DRSRGIFFTDQDWSLPGVLPAVSGGIHVHWPALTEIFGDDSVLQFGGGTLGHPWGNAPG
DRSRGIYFTQDWCSPGVMPVASGGIHVHWPALVEIFGDDACLQFGGGTLGHPWGNAPG
NLPYGIFFEMTWASLRKCPVASGGIHCQMHLVHYLGDDVVLQFGGGTIGHDPGIQAG
.: ***. * * * : .***** : * * * : *** *****.*** * *

AVANRVALEACVQARNEGRDLAV--EGNEIIREACKWSPELAAACEVWKEITFNFTIDK
AAANRVALEACTQARNEGRDLAR--EGGDVIRSACKWSPELAAACEVWKEIKFEFDTIDK
ATANRVALEAMILARNEGADYFNSDIGPQILRNAAKTCGPLQTALDLWKDISFNYTSTD
* ***** *

LDGQE-----
L-----

Conclusion: comparison of full-length sequences to determine if a given antibody can be suitable can be misleading for antibodies made for short synthetic peptides or protein fragments.

Sequence-Predicted antibody reactivity

sp P0D018 LHR32_CHLRE Mp2g13460.1	MQTKELNNGRLAMIAIAAFVAQELVEQTEIFEHLALRFEKEAILELDDIE LKVKEKNGRLAMTAMLGFFVQAIVTGKGPVQNLYDHLANPQINNAWAYA ::.**:***** *: .*.* :* . :.* :: : * :
sp P0D018 LHR32_CHLRE Mp2g13460.1	RDLGLPVTPLPDNLKSL TNFVPRS----- ::

Confirmed reactivity: *Chlamydomonas reinhardtii*

Overall sequence conservation to Marchantia polymorpha (Mp): low

Immunogen sequence conservation: 100 %

Misconception: Knowing immunogen sequence will allow to identify any cross reacting bands in Western blot

A sequence alignment is not predicting how a denatured protein fragment will physically present itself to an antibody on a PVDF or nitrocellulose membrane.

Mass spec analysis or **sequencing** are the methods to confirm which proteins are detected by an antibody as cross-reacting bands.

Predicted vs. demonstrated cross-reactivity

Confirmed reactivity: *Arabidopsis thaliana*, *N. benthamiana*

Predicted: *Vitis vinifera*

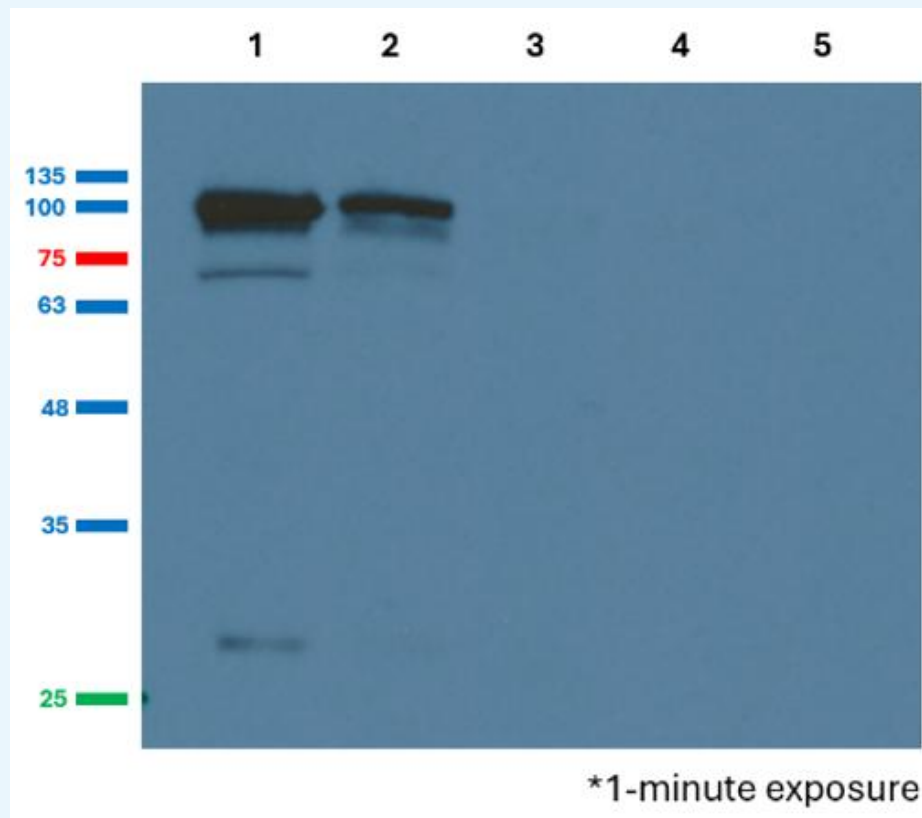
Experimental data

Confirmed reactivity: *Arabidopsis thaliana* (1), *Nicotiana benthamiana* (2)

Predicted reactivity: *Vitis vinifera* (3,4,5)

Immunogen sequence conservation: 100 %

Experimental result: negative



Misconception: Antibody production is a rather standard process, independent upon a protein.

Agrisera Antibody Production Guide

Antibody production checklist:

- **Protein Target Evaluation** protein abundance < 10 ppm will require more effort ([ProteinAbundance Database](#))
- **Unique Immunogen production** and QC >90 % purity
- **Application:** Define the intended use (WB, IHC/IF, ELISA, IP, flow cytometry).
- **Pre-immune serum screening** (especially important for plant proteins)
- **Production & timeline:** What is going to be delivered? An immunogen affinity purified antibody of known concentration with titer (concentration) confirmed in ELISA test.
- **Expected antibody yield:** from 2 to 20 mg of specific antibody/20 ml of serum.
- **Your own validation:** grow/collect required material in advance including necessary controls.

How much do you know about the protein you aim to detect with your antibody?

Protein checklist:

- Part of a larger protein family?
- Biochemical properties
 - Isoelectric point
 - Solubility
- Organ/tissue localization
- Cellular localization
- Expression level ([PaxDb](#))

First criteria: amount of target protein in the tissue

Protein Abundance Database

Arabidopsis thaliana protein

Q8RVQ9 : Histone H3-like centromeric protein HTR12; Histone H3-like variant which exclusively replaces conventional H3 in the nucleosome core of centromeric chromatin at the inner plate of the kinetochore. Required recruitment and assembly of kinetochore proteins, mitotic progression and chromosome segregation. May serve as an epigenetic mark that propagates centromere identity through replication and cell division (By similarity):

in: UniProt

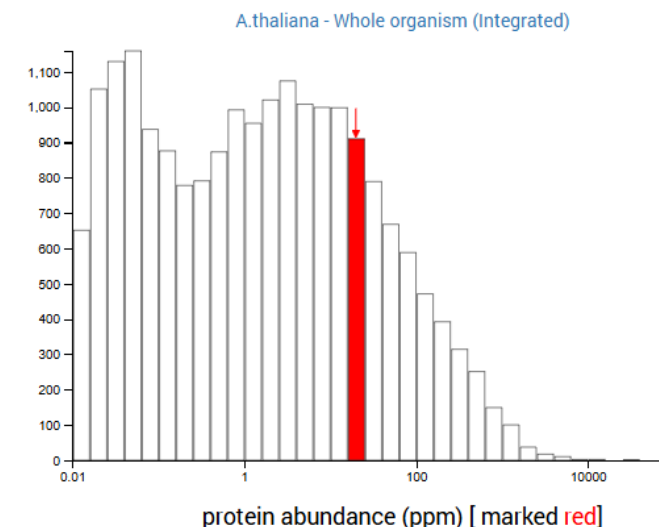
abundance information

Search dataset/tissue

Dataset	Tissue type	Abundance	Dataset score 
A.thaliana - Seed, Seed (Mergner et al, Sci Data 2020)	seed	36.5 ppm, 4573. out of 11123	11.7
A.thaliana - Silique (Integrated)	silique	57.8 ppm, 3199 / 13305 [top 25 %]	11.5
A.thaliana - Silique, Silique (Mergner et al, Sci Data 2020)	silique	36.1 ppm, 4746. out of 11123	11.3
A.thaliana - Seed (Integrated)	seed	20.4 ppm, 5125. out of 11934	11.1
A.thaliana - Rosette (Integrated)	rosette	1.58 ppm, 9349 / 10947 [bottom 25 %]	9.2

« < ... 1 2 3 4 5 ... > »

Protein Abundance Histogram



For a target protein with an abundance of <10 ppm, detection is going to be more challenging.

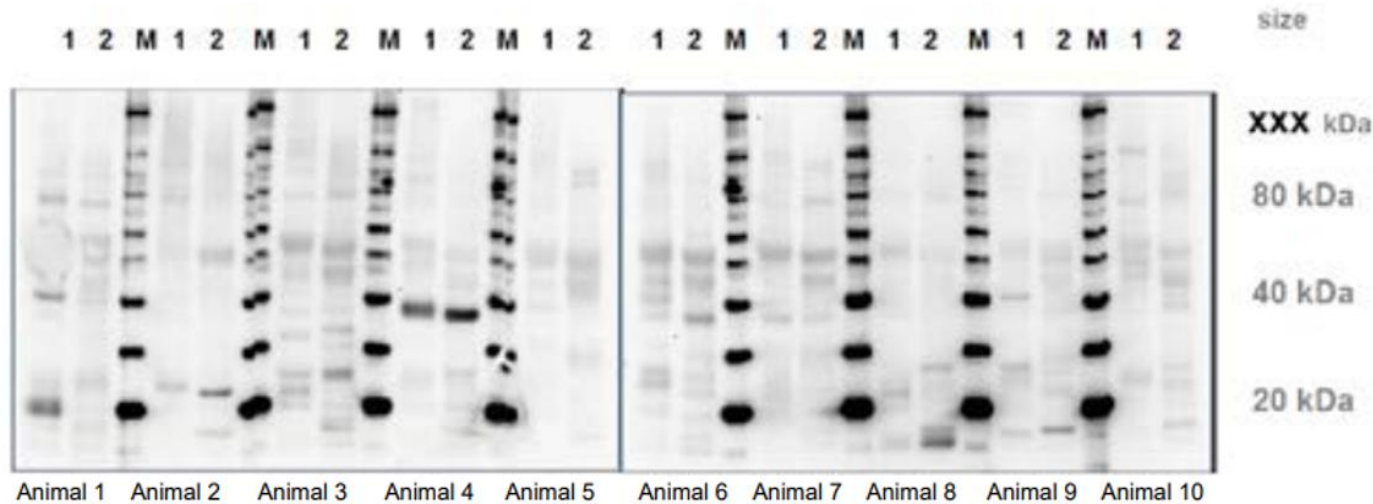
Before immunization starts: serum pre-screening

Sample load: 5-10 µg/well (1- *Arabidopsis thaliana*, 2- *Hordeum vulgare*).

Blocking: 5-10% non-fat milk.

Primary antibody (pre-immune serum) dilution: 1: 1 000 - 1: 10 000, incubation 1h/RT.

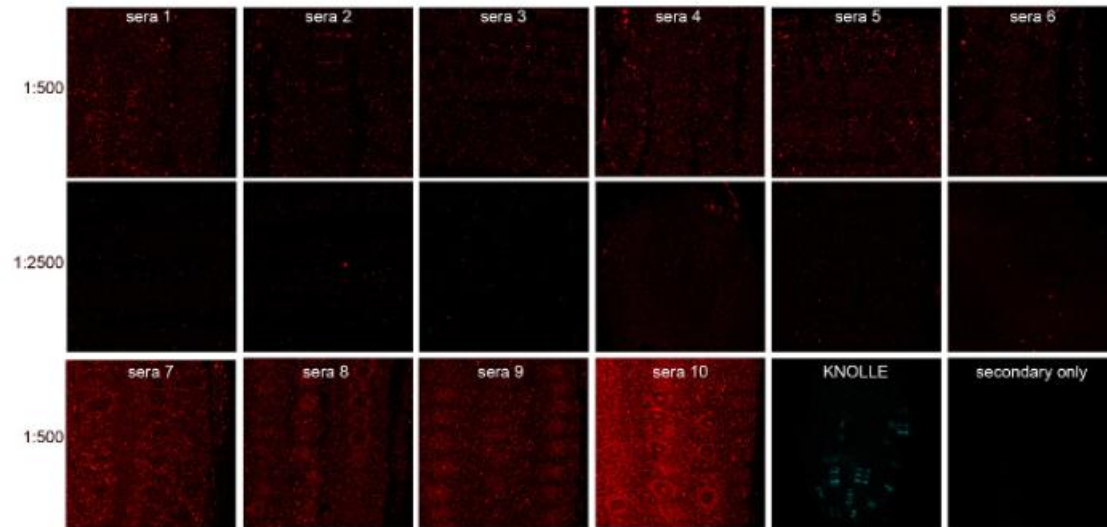
Secondary antibody dilution: 1: 25 000, incubation 1h/RT (Goat anti-Rabbit, AS09 602)



Check out Agrisera's
educational poster about
antibody production

[To poster](#)

Before immunization starts: serum pre-screening



In the above example, serum collected from 10 different not immunized rabbits (pre-immune serum) were screened by immunofluorescence of 5-day-old roots of *Arabidopsis thaliana*, ecotype Col-0 (WT).

For Immunolocalization:

Pre-immune serum dilution: 1:500,

Secondary antibody dilution: 1:2500,

CY5-coupled donkey anti-rabbit dilution: 1:300,

Positive control: polyclonal antibody, rabbit anti-KNOLLE at a dilution of 1:4000 (Lauber MH et al. 1997. J Cell Biol 139: 1485–1493),

Negative control: incubation with secondary antibody without primary antibody.

Courtesy of Dr. Anna Gustavsson and Prof. Markus Grebe, Umeå Plant Science Centre, Sweden.

Antibody production and validation process is outlined in the Agrisera Antibody Production Poster, which can be viewed [here](#).



**Pre-immune screening
procedure**

[Described here.](#)

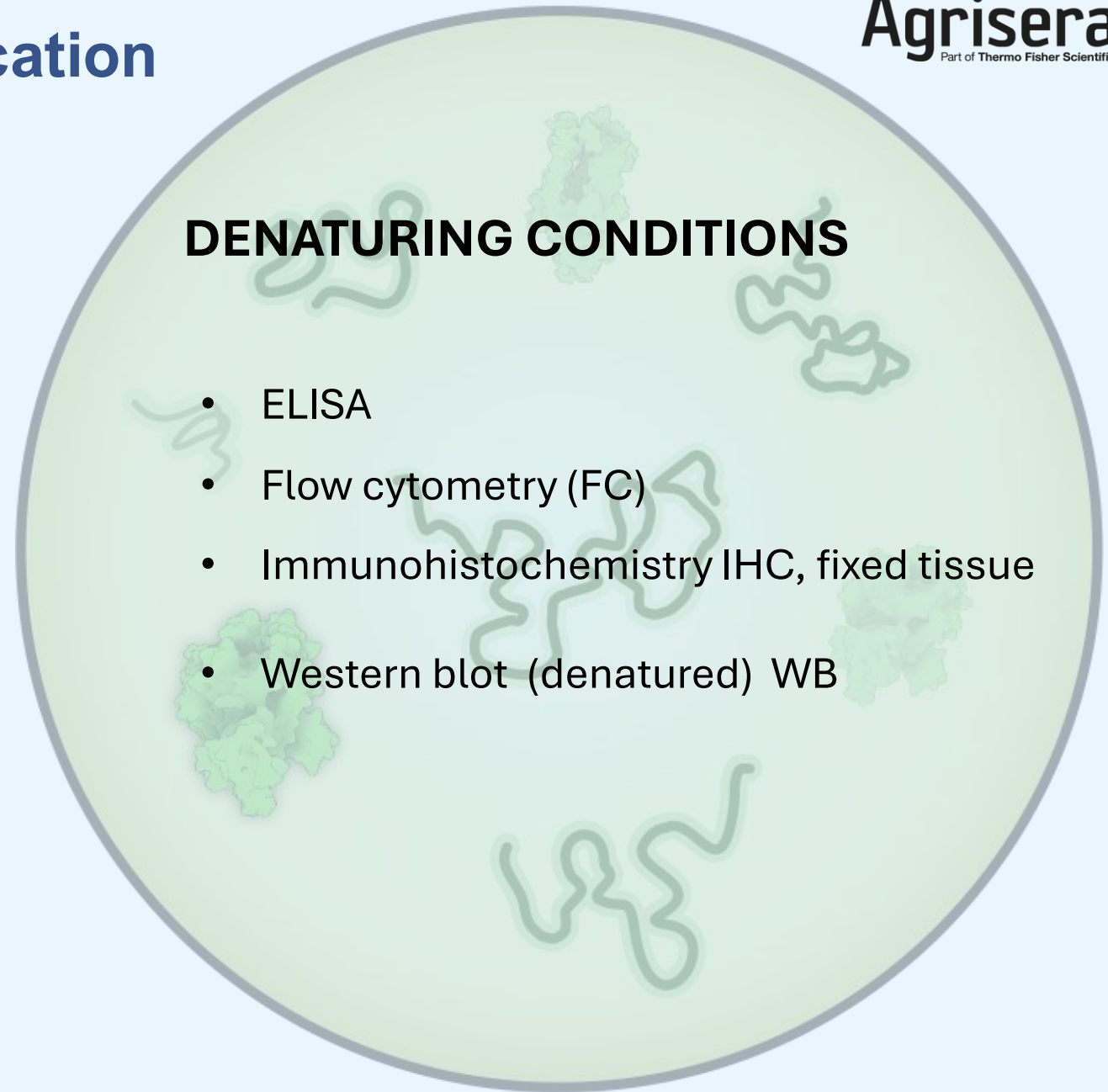
How to match antibody to application

NON-DENATURING CONDITIONS

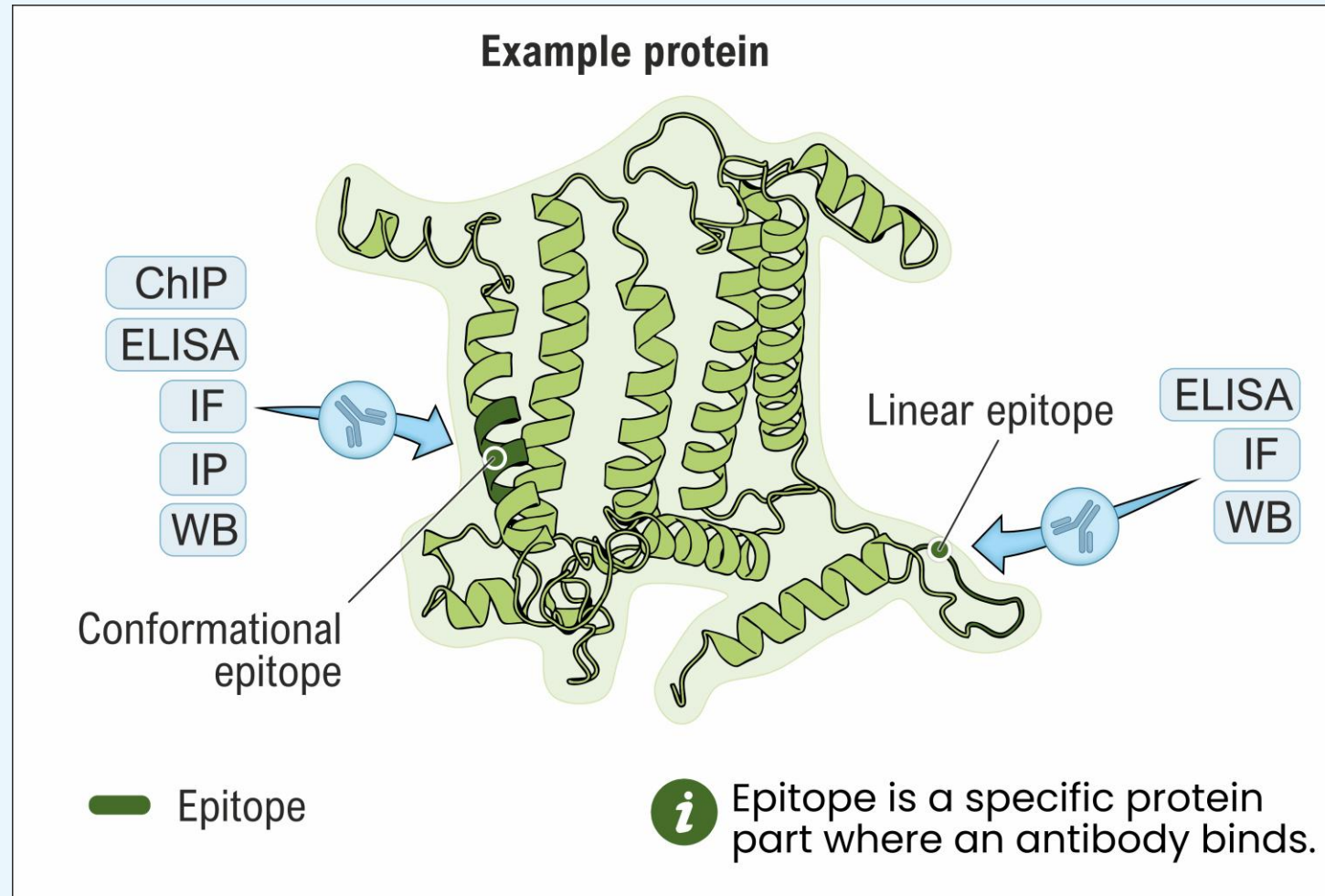
- ELISA
- Flow cytometry (FC)
- Immunoprecipitation (IP)
- Western blot WB

DENATURING CONDITIONS

- ELISA
- Flow cytometry (FC)
- Immunohistochemistry IHC, fixed tissue
- Western blot (denatured) WB

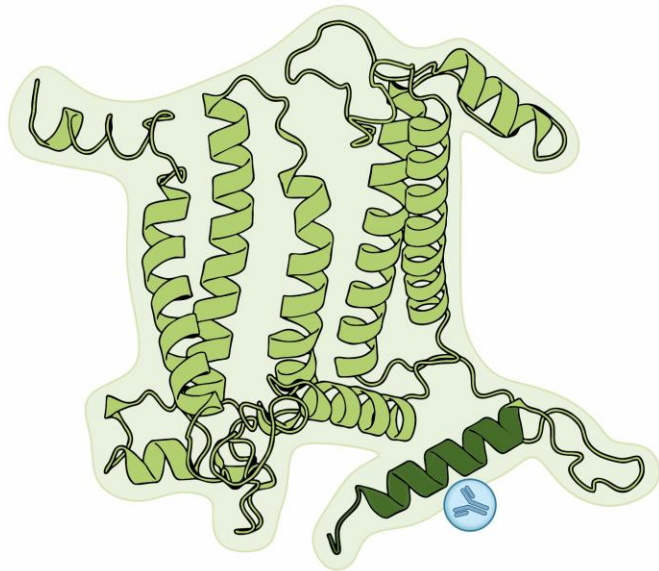


Epitope exposure in selected applications

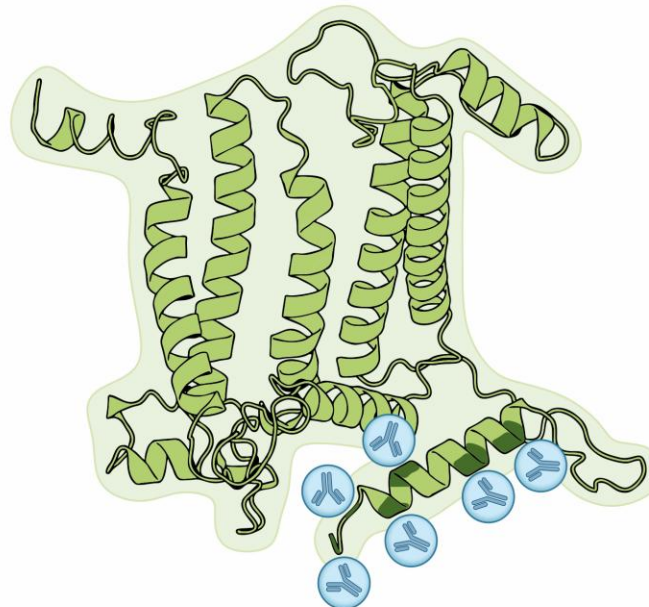


Misconception: antibodies give a lot of cross reacting bands in WB in denatured conditions, therefore I can not use them in immunoprecipitation (IP) in native conditions.

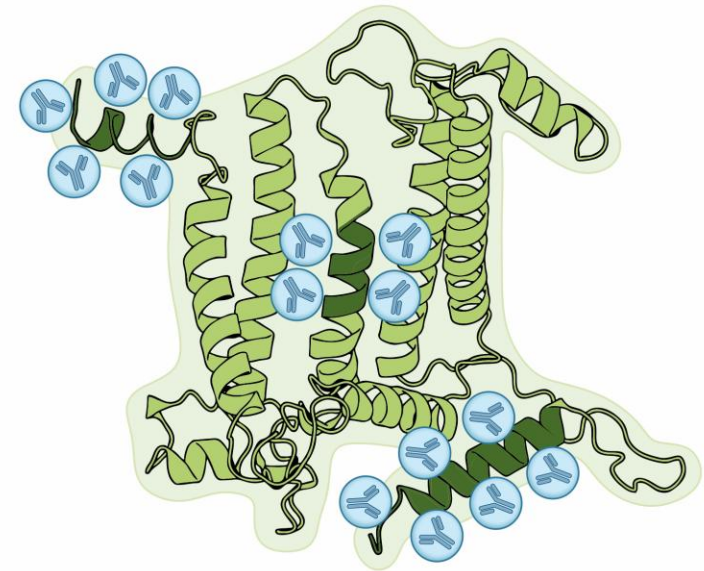
Misconception: monoclonal antibodies are superior to polyclonal



Monoclonal

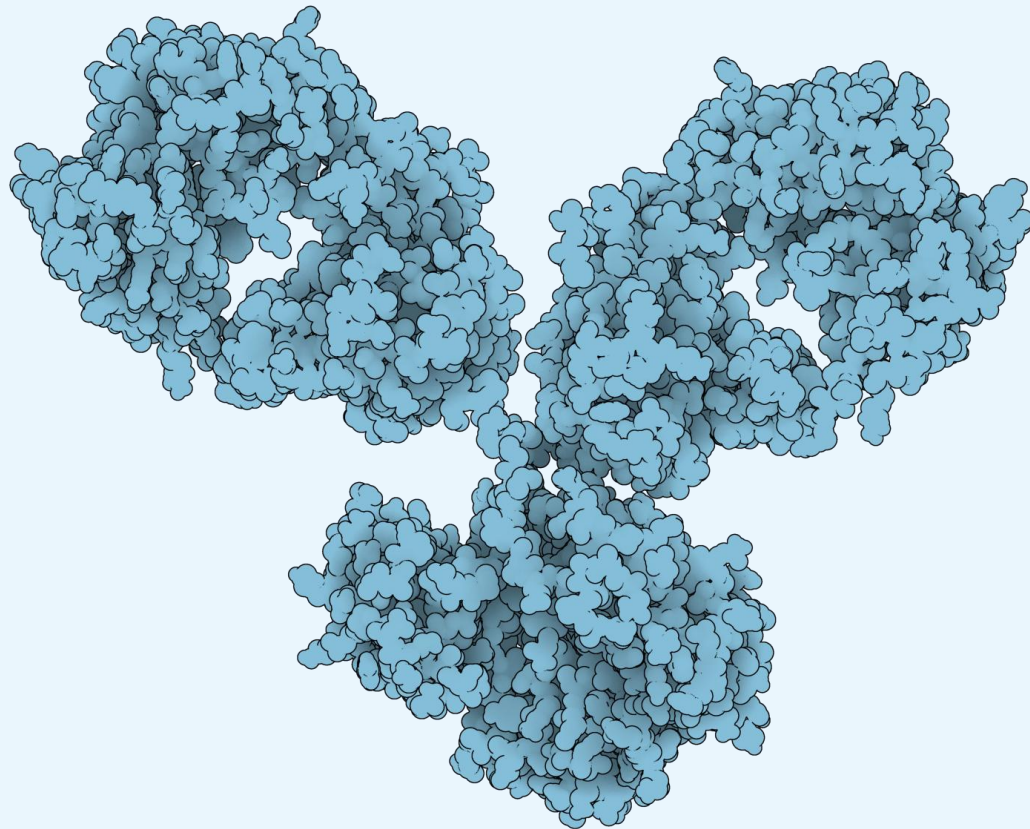


Polyclonal
(anti-short peptide)



Polyclonal
(anti-whole protein)

Recombinant antibodies & multiconals



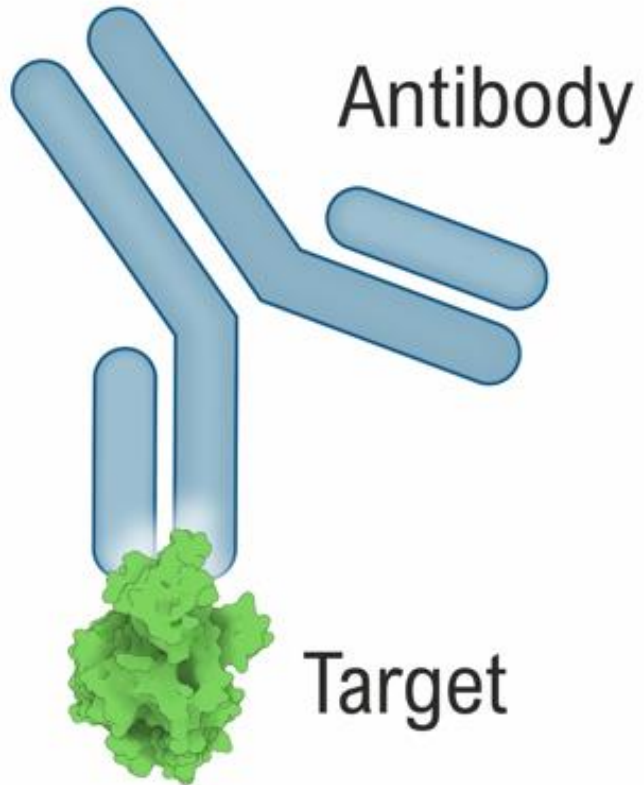
Antibodies recognizing specific epitopes are selected, sequenced, cloned and overexpressed. They are called **recombinant antibodies**.

Multiconals consist of a pool of recombinant antibodies recognizing chosen epitopes.

Advantages:

- Minimal batch to batch variation
- Ease of production in large quantities
- Disadvantage: high cost

What is antibody validation?



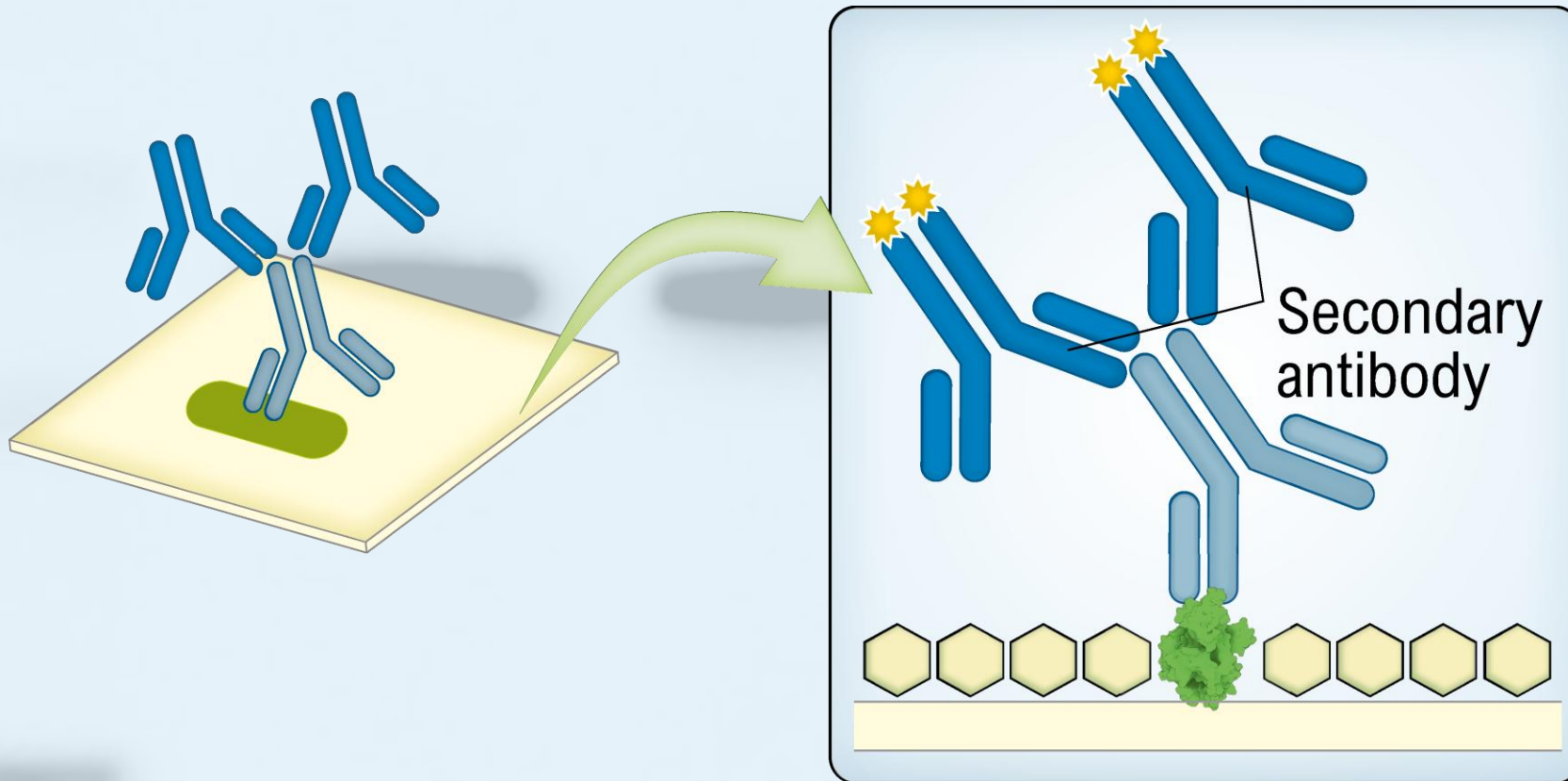
Experimental confirmation that an antibody binds to the correct target protein in a given application.

Specific

Selective

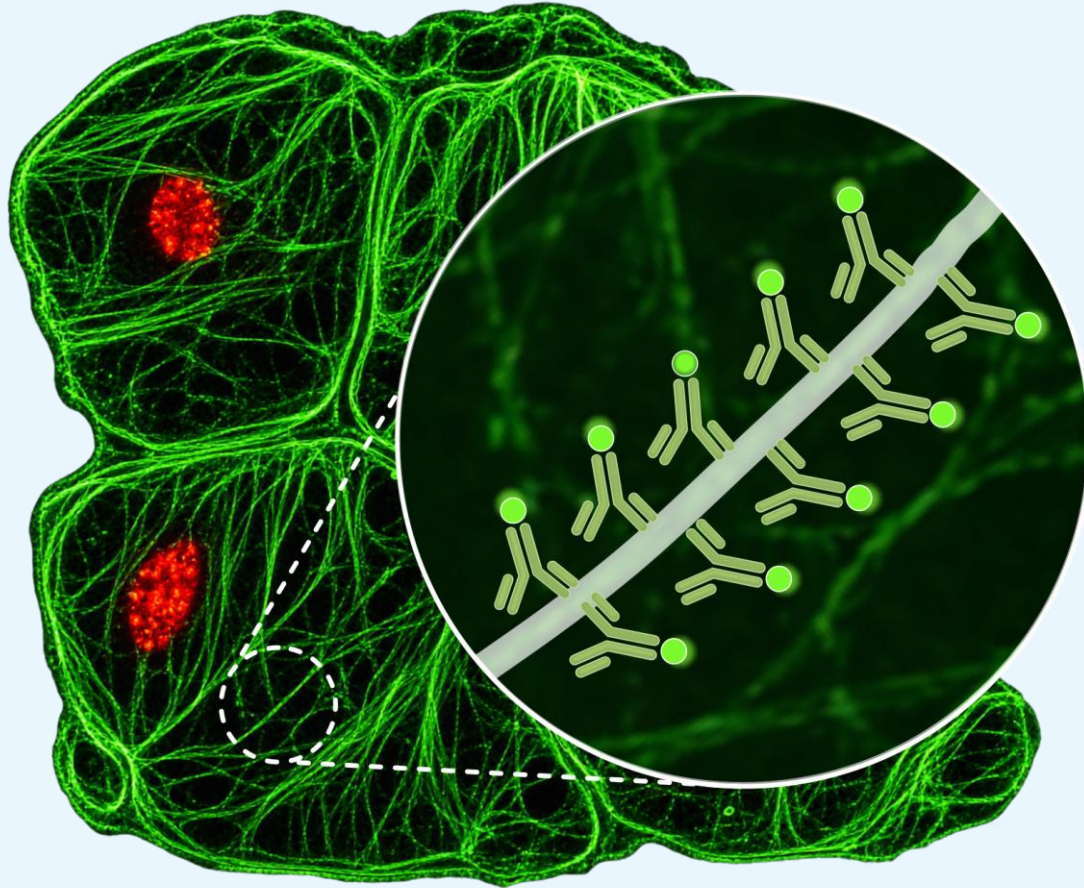
Reproducible

Each technique requires a separate validation with appropriate controls

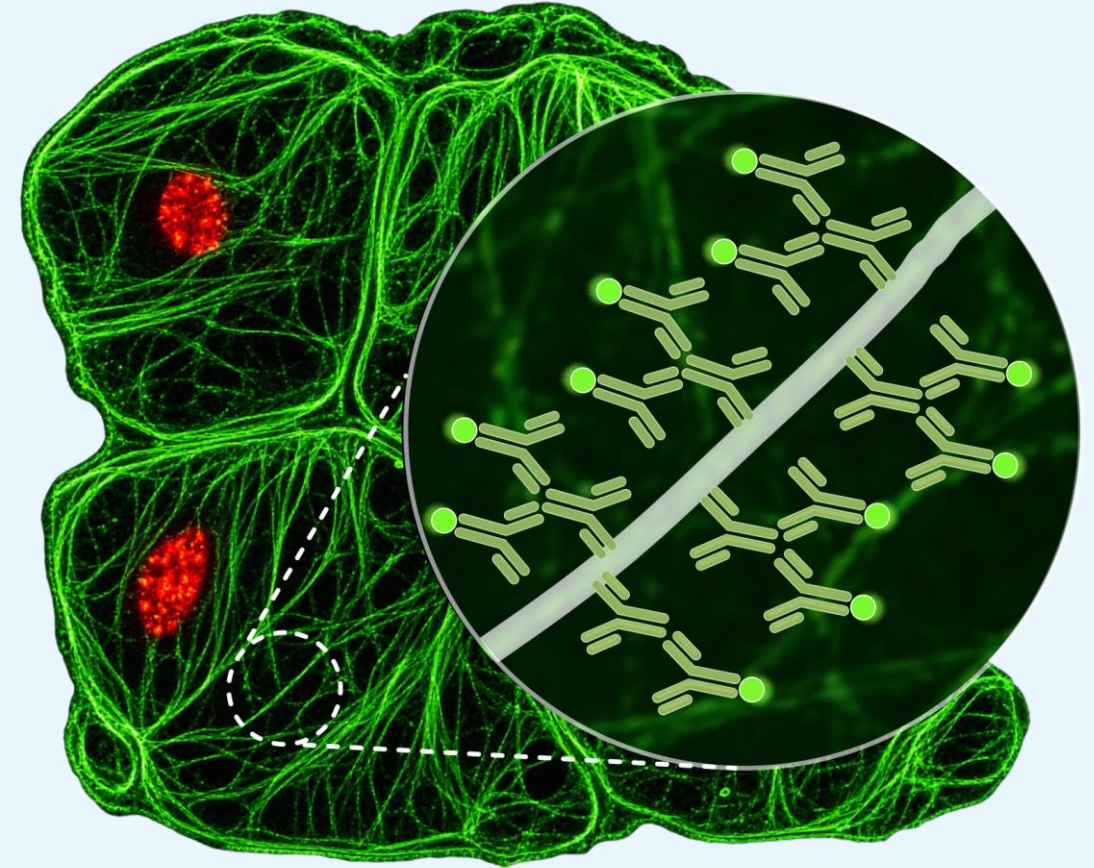


[Antibody validation explained](#)

Antibody binding in immunofluorescence



One step immunofluorescence (IF)

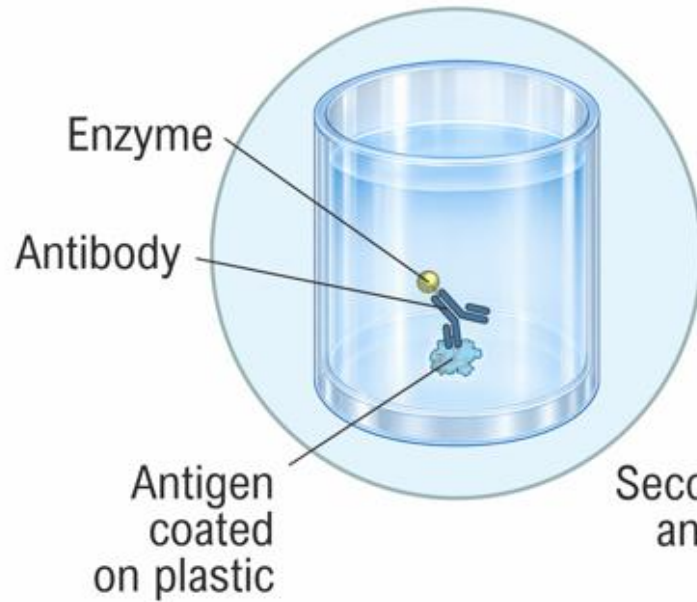


Two step immunofluorescence (IF)

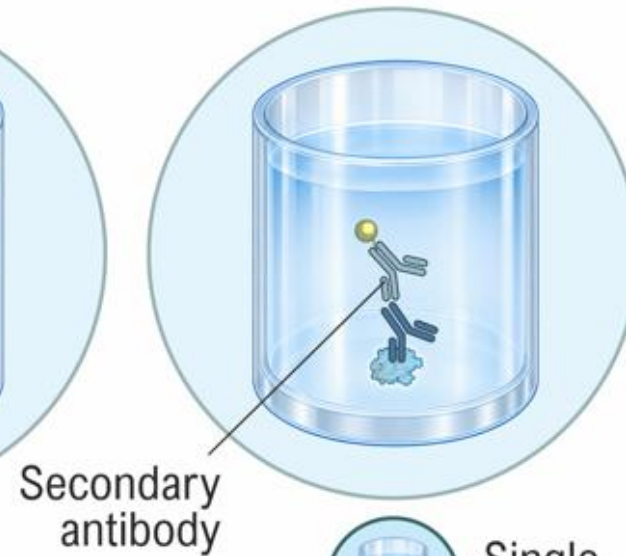
ELISA assay (Enzyme-Linked-Immunosorbent-Assay)

Types of ELISA

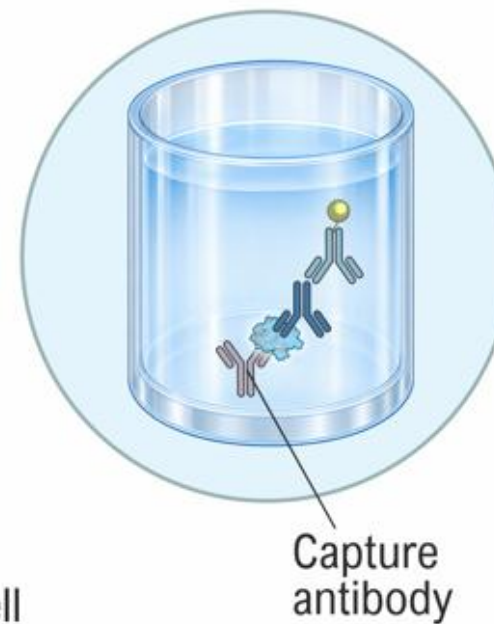
Direct ELISA



Indirect ELISA



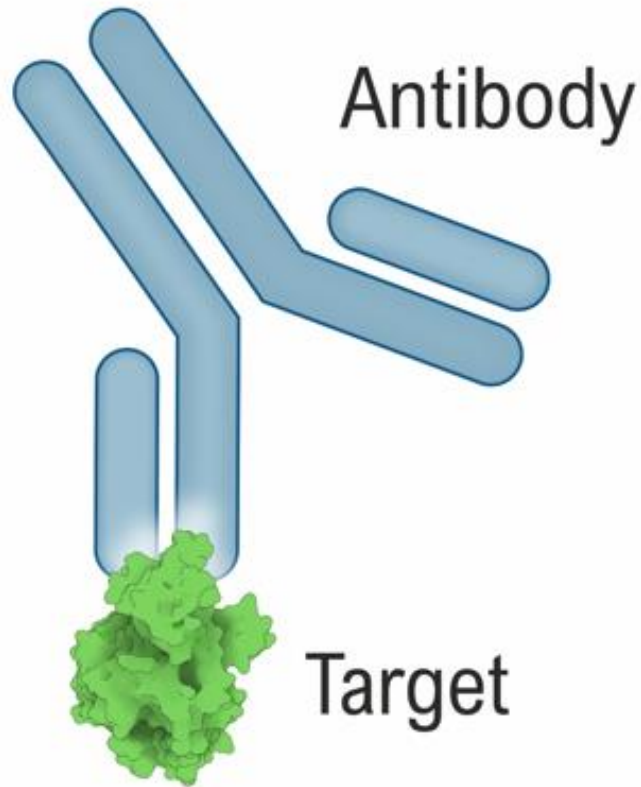
Sandwich ELISA



Single ELISA well



Immunoprecipitation (IP) – method to isolate and concentrate specific protein using antibodies while coIP is used to study protein-protein interaction



Recognized epitopes: native, accessible and surface exposed.

Antibody validation strategies for various applications

- **Specificity:** Strong evidence that the antibody recognizes the intended target.
- **Controls:** Positive/negative controls, knockout/knockdown controls, isotype controls where appropriate, and secondary-only controls.
- **Orthogonal validation:** Confirmation using an independent method or reagent (e.g., genetic manipulation, mass spectrometry, or a second antibody).
- **Biological context:** Appropriate cell/tissue type, target abundance, localization, and relevant stimulation or treatment conditions.
- **Performance characteristics:** Sensitivity, signal-to-noise, reproducibility, dynamic range, and optimal antibody concentration.
- **Protocol-specific factors:** Fixation, antigen retrieval, denaturation, permeabilization, blocking, and other conditions that can affect antibody performance.
- **Reproducibility:** In independent experiments & well documented protocols.

Ideal Western blot result: band of correct MW is gone in the KO mutant and present in the overexpressor

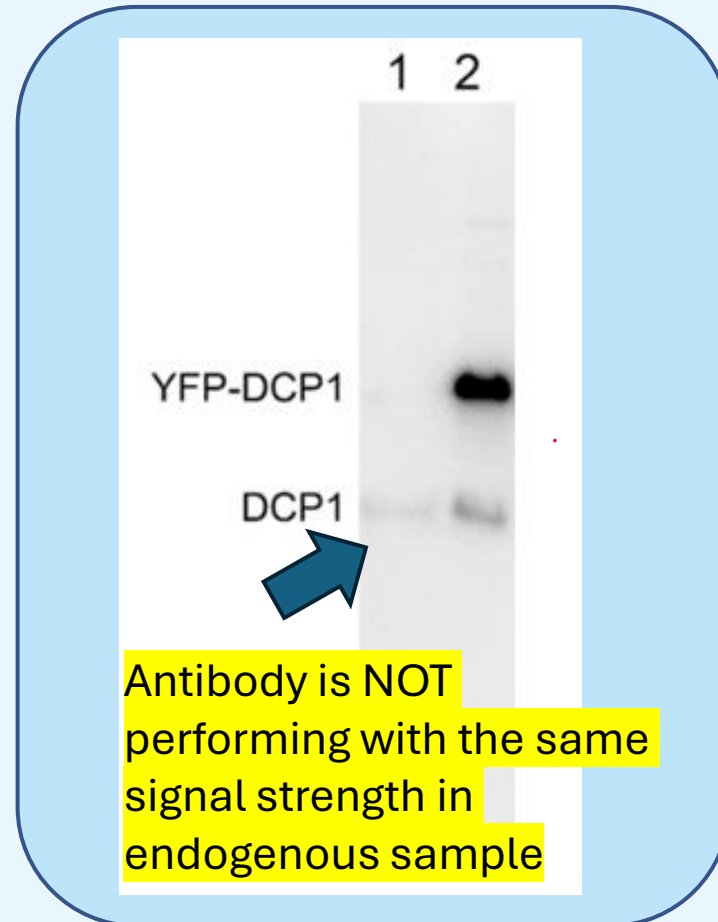
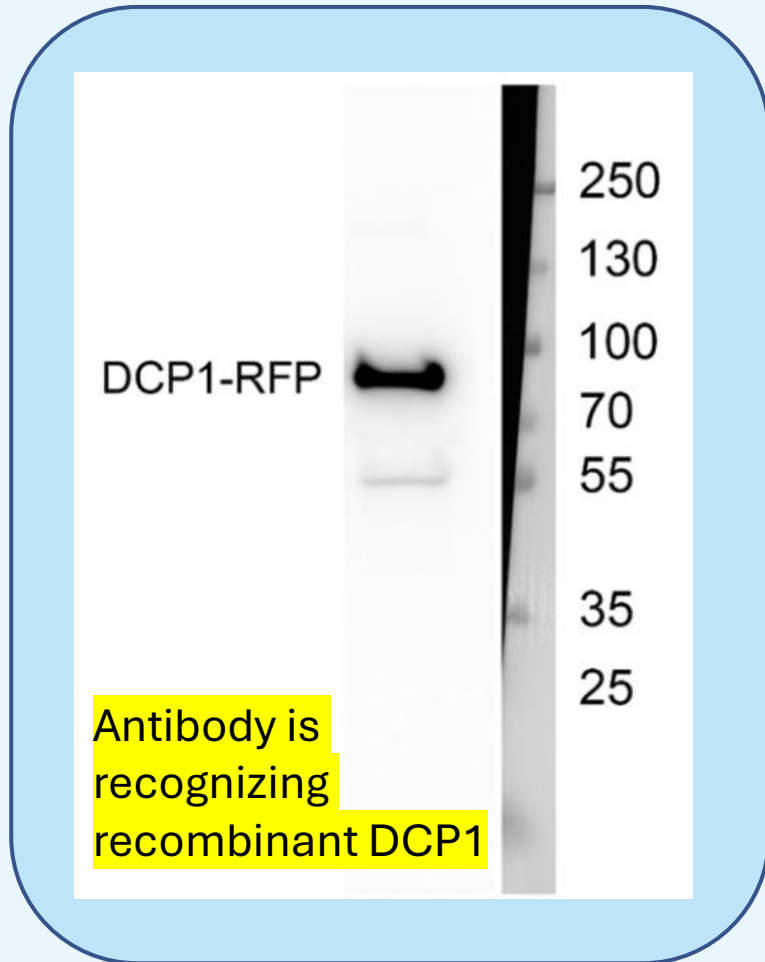
Samples:

- 1 - *A. thaliana* WT
- 2 - *A. thaliana* WT
- 3 – Knockout mutant (KO)
- 4 – Overexpressing mutant

Target
protein



Misconception: Antibody reactivity to recombinant protein is a solid proof that given antibody is going to recognize endogenous protein.



Samples:

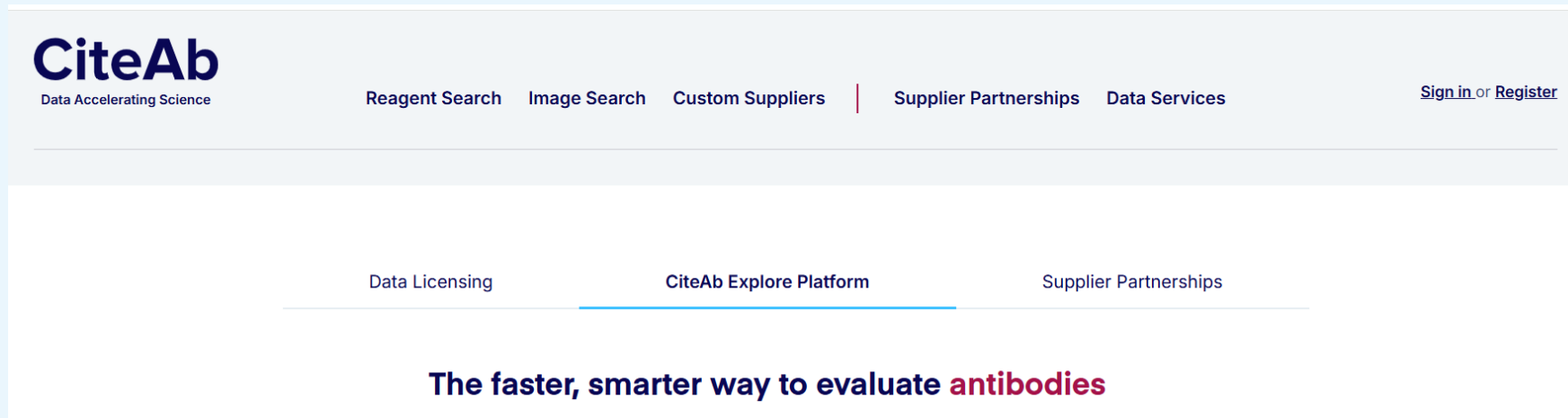
- 1 - *A. thaliana* WT
- 2 - YFP-DCP1 overexpressing *A. thaliana*

How to find validated antibodies



<https://www.antibodypedia.com/>
5 mln reviewed antibodies (5 mln antibodies to human proteins)

<https://www.biocompare.com/>



<https://www.citeab.com/>

Basic information to be provided on the antibody product information sheet

Example: Anti-AGO1, AS09 527

Immunogen is not an epitope

Immunogen:	KLH-conjugated, N-terminal peptide of <i>Arabidopsis thaliana</i> AGO1 O04379, At1g48410
Host:	Rabbit
Clonality:	Polyclonal
Purity:	Immunogen affinity purified serum in PBS pH 7.4.
Format:	Lyophilized
Quantity:	50 µg
Reconstitution:	For reconstitution add 50 µl of sterile water
Storage:	Store lyophilized/reconstituted at -20°C; once reconstituted make aliquots to avoid repeated freeze-thaw cycles. Please remember to spin the tubes briefly prior to opening them to avoid any losses that might occur from material adhering to the cap or sides of the tube.
Tested applications:	Chromatin Immunoprecipitation (ChIP), Immunofluorescence (IF), Immunolocalization (IL), small-RNA-IP-Seq, Western blot (WB)
Recommended dilution:	2 µg (ChIP), 1: 200 (IF), (IL), small-RNA-IP-Seq, 1 : 5000-1 : 10 000 (WB)
Expected apparent MW:	116.4 130 kDa

Basic information to be provided on the antibody product information sheet

Example: Anti-AGO1, AS09 527

REACTIVITY

Confirmed reactivity:

Arabidopsis thaliana, Capsella rubella, Capsicum annuum, Hyacinthus orientalis, Lotus japonicus, Nicotiana benthamiana

Predicted reactivity:

Brassica napus, Brassica pekinensis, Cucumis sativus, Glycine max, Malus domestica, Pisum sativum, Ricinus communis, Solanum lycopersicum, Solanum tuberosum, Vitis vinifera

Species of your interest not listed? [Contact us](#)

Not reactive in:

Chlamydomonas reinhardtii, Medicago sativa, Salvia sp., Spinacia oleracea, Oryza sativa, Phaseolus vulgaris, Triticum aestivum, Zea mays

Mammals

Basic information to be provided on the antibody product information sheet

Example: Anti-AGO1, AS09 527

APPLICATION EXAMPLES

ADDITIONAL INFORMATION

BACKGROUND

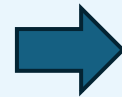
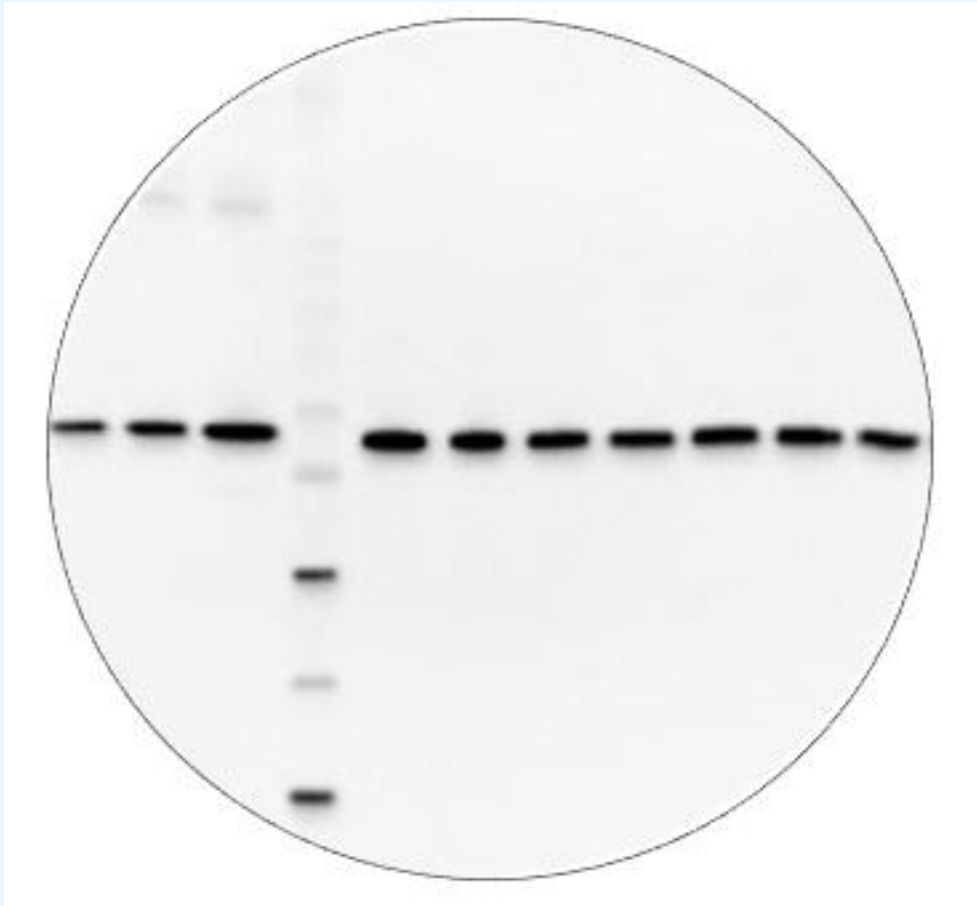
PRODUCT CITATIONS

Antibody Product information sheet should be used as a guide on how to use an antibody.



[Product information sheet explained in details.](#)

Misconception: A band at correct MW detected in Western blot is a solid proof that antibody is binding to a protein I want to visualize.



**Protein load: 0.1 - 0.5 µg/well
 10^{11} to 10^{12} individual proteins tightly
packed into a single band.**

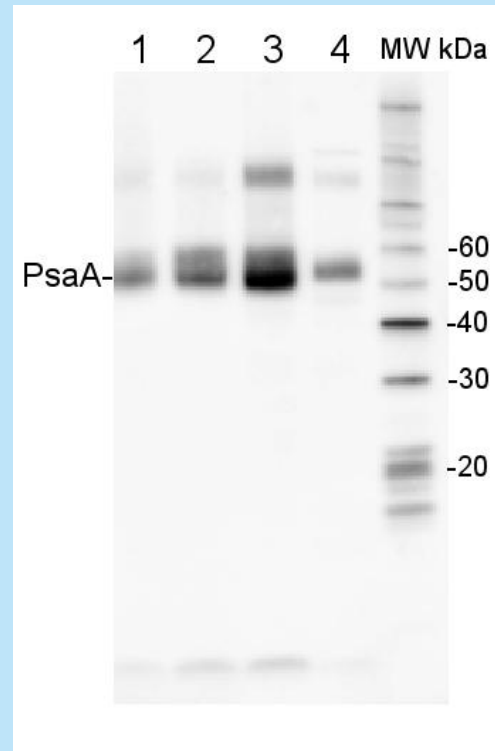
Calculated and apparent MW is not always the same

Example: PsaA | PSI-A core protein of photosystem I

Length: 751 amino acids

Calculated MW: 83 kDa

Apparent MW: 55-60 kDa



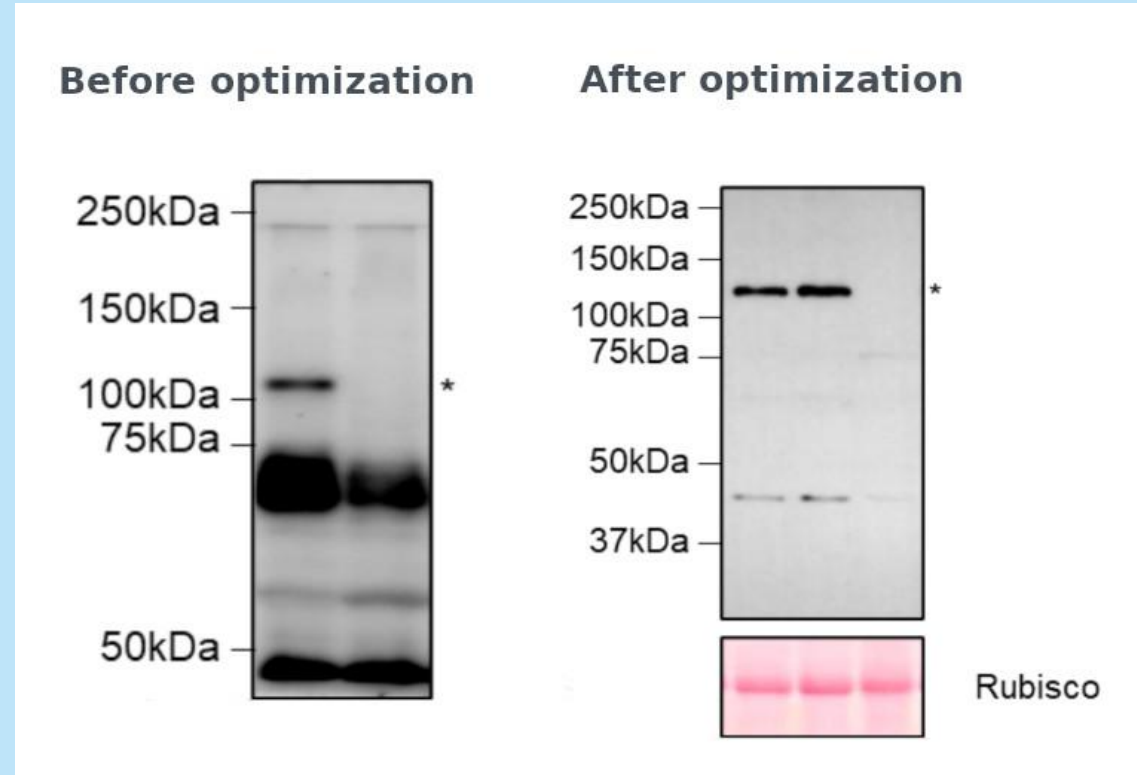
Modifications which may influence Protein mobility on SDS gel:

- N or C-terminal processing
- Post-translational modifications (PTMs)
- Amino acid composition (>10 kDa)
- Aggregation or proteolytic cleavage
- Epitope tags
- SDS-PAGE conditions



Why is calculated and apparent MW of detected protein different?

Misconception: Using more of a primary antibody will result in improved signal

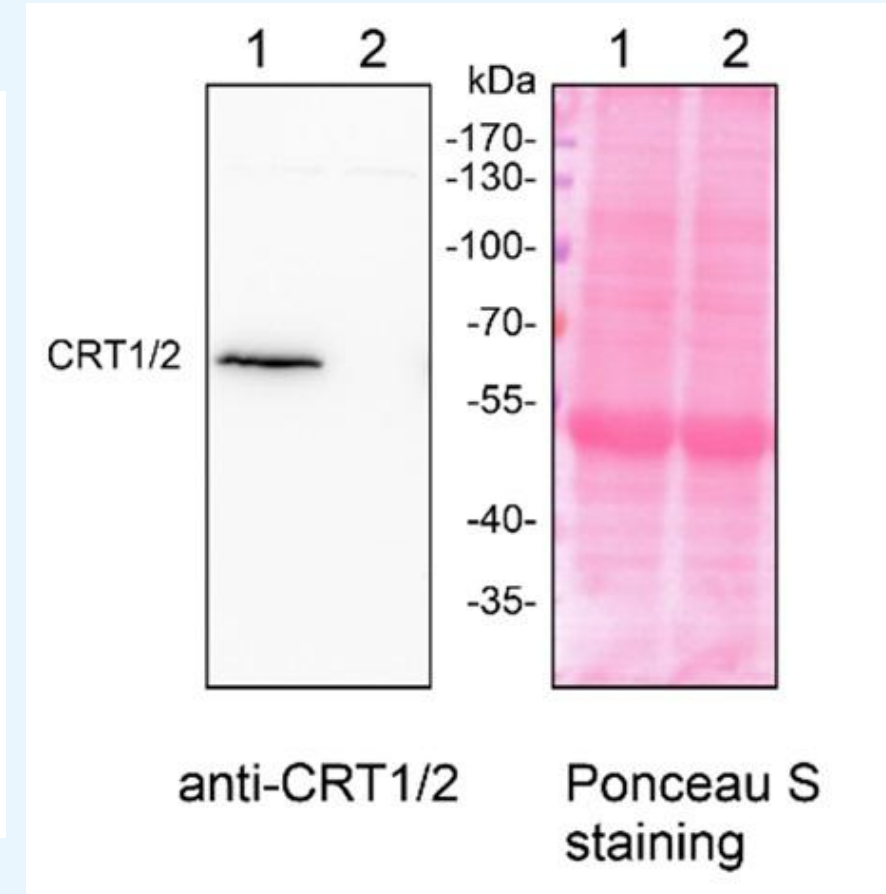
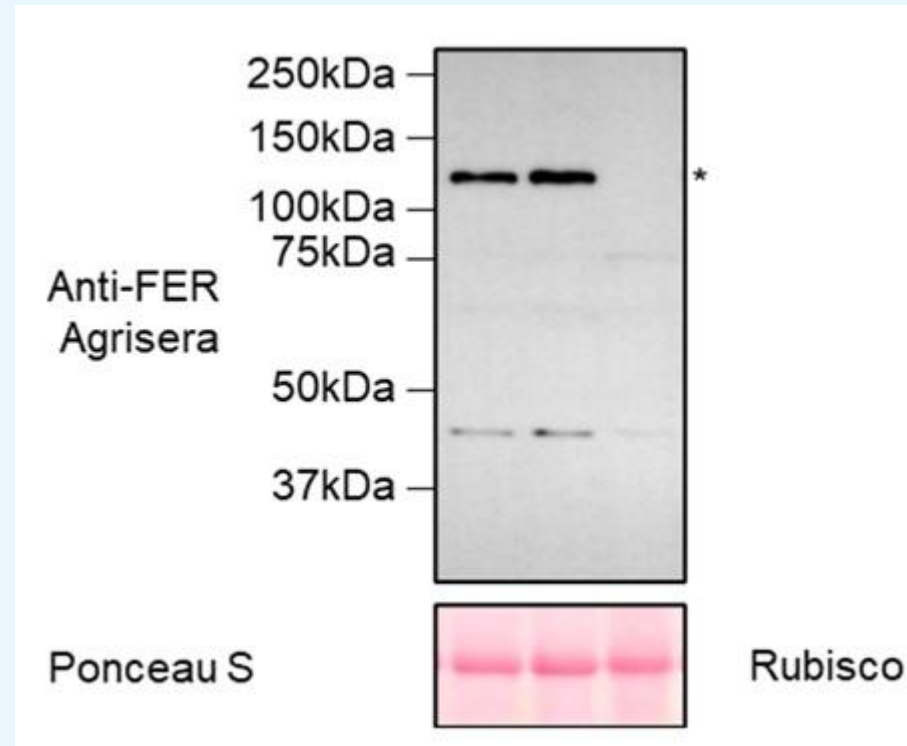
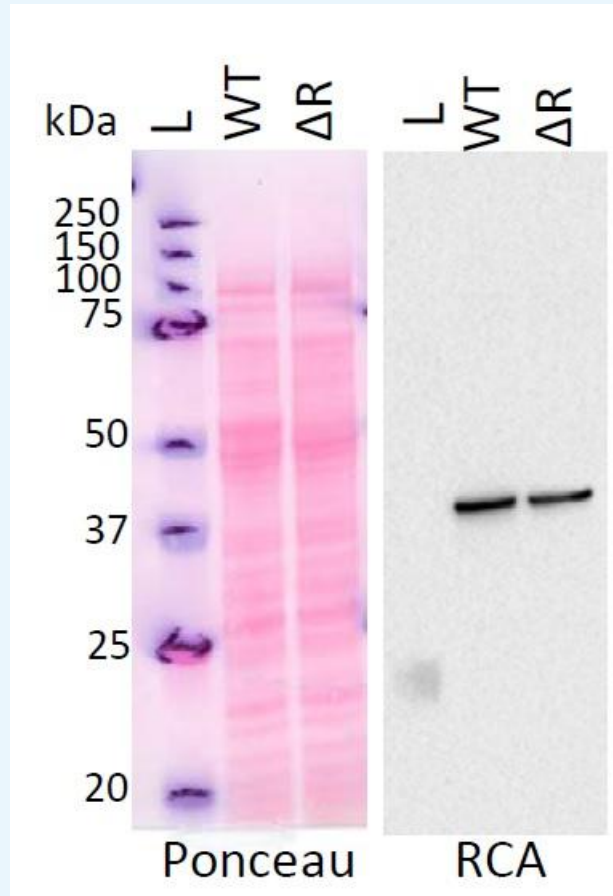


Primary antibody:

1: 1000

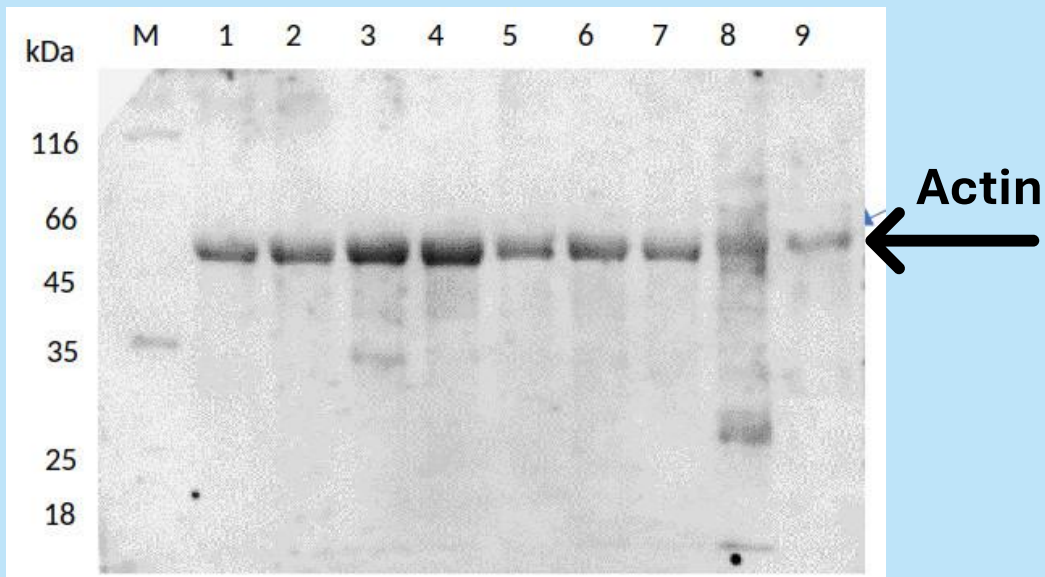
1: 4000

Misconception: one and the same protocol can be applied to each protein-antibody pair



Misconception: Anti-actin and anti-tubulin are universal antibodies and perfect controls for all experimental conditions.

Metal stress



Other possible internal controls:

- Total protein (express fmol target protein/ μ g total protein)
- Dry mass
- Total chlorophyll (express fmol target protein/ μ g chlorophyll)
- Cells (express fmol target protein/cell)
- Total nitrogen (express fmol target protein/fmol N)
- Experiment specific ratios of particular proteins (ex. fmol PsbA/fmol RbcL)

Courtesy of MSc, Karla Košpić, University of Zagreb, Faculty of Science Department of Biology, Croatia.

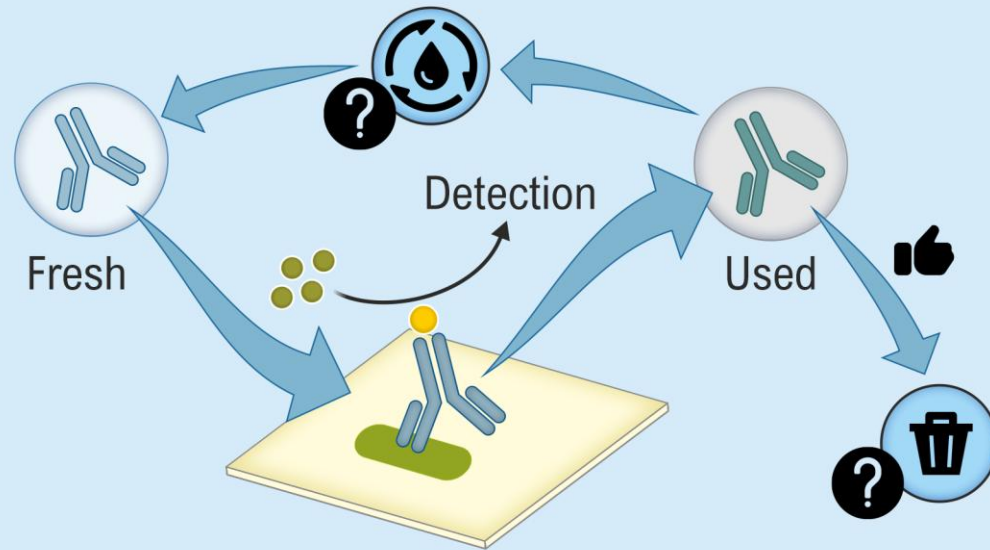
Misconception: primary antibodies can always be re-used

1 x Reused

20 x Reused

Never re-used

Can Primary Antibodies be Re-Used?



Generally not recommended, especially for quantitative experiments



Recommended in most cases to avoid signal loss



Re-using of primary antibodies explained in further details

Misconception: Antibodies can easily be compared between each other

Q: I work with a not fully characterized primary antibody to a protein of low expression, and I noticed that I get a signal with actin, but not with that primary antibody.

Why is that?



Note:

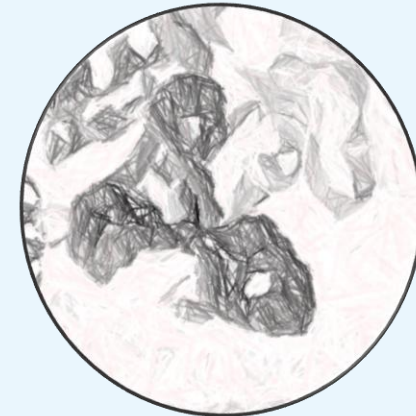
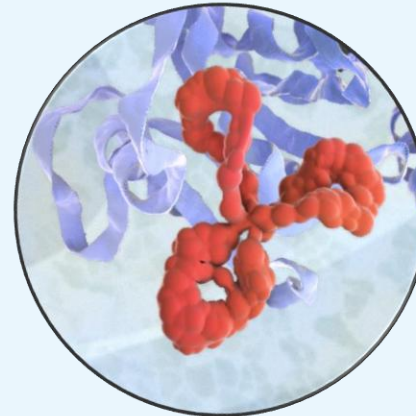
Actin, a high-abundance protein, and a target protein of low expression, cannot be directly compared!

[Check protein abundance in PAXdb](#)



Are monoclonal antibodies better, compare to polyclonal antibodies?

[Find out!](#)



Misconception 1: This antibody is so well published, therefore it must work for my samples and experimental set up too.

A published antibody is NOT automatically validated.

Misconception 2: Specific Antibody Catalogue numbers are not necessary to be included in the publication.

Antibody name, product number, supplier

Example:

Anti-RbcL | Rubisco large subunit, form I (rabbit), AS03 037,
Agrisera

Beat the antibody misconceptions in Agrisera Quiz and get a chance to win a free antibody



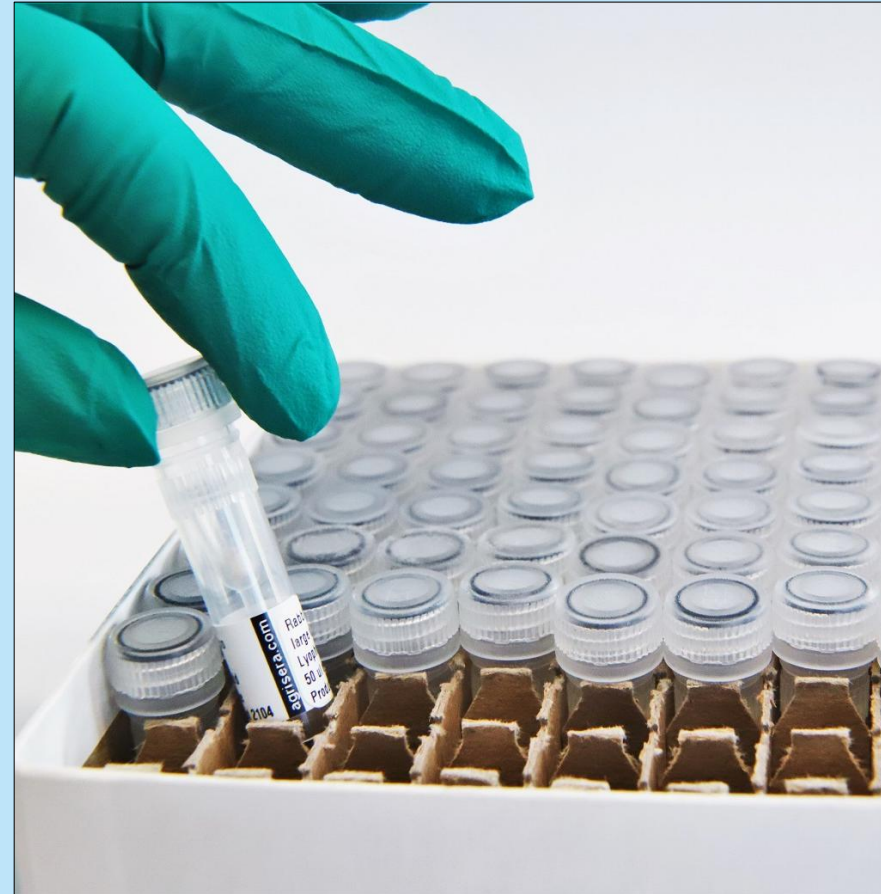
<https://agrisera-antibodies.typeform.com/260924>

>100 free antibodies

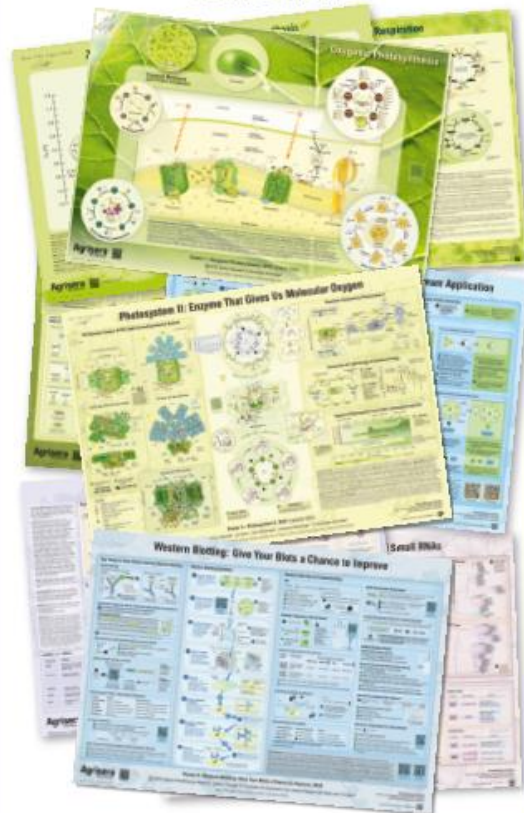
- No limit of provided antibodies
- In return for results
- Secondary antibodies included



[Check out the list of free antibodies, updated weekly](#)



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Thank you!

Your questions are always welcome!