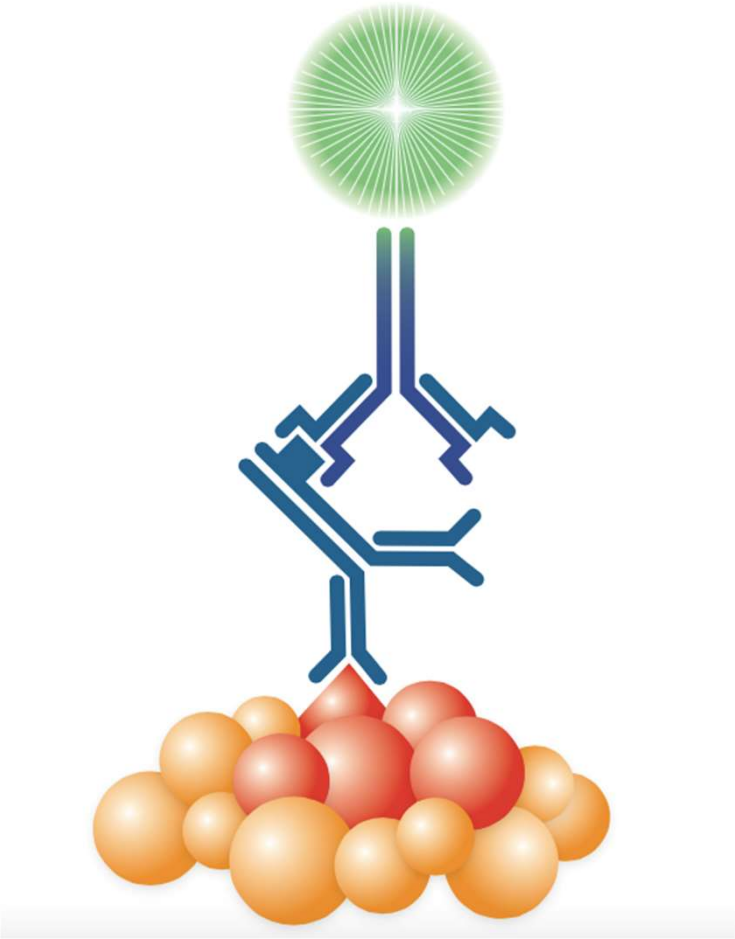


Western blotting

Olivia Tjahjono
Tsend-Ayush Batbold

https://www.drugdiscoveryonline.com/doc/western-blotting-principles-and-methods-handbook-0001
6/14

What is western blotting?

Western blotting (WB) is an analytical technique to detect specific protein molecules among a mixture of proteins.

Procedure

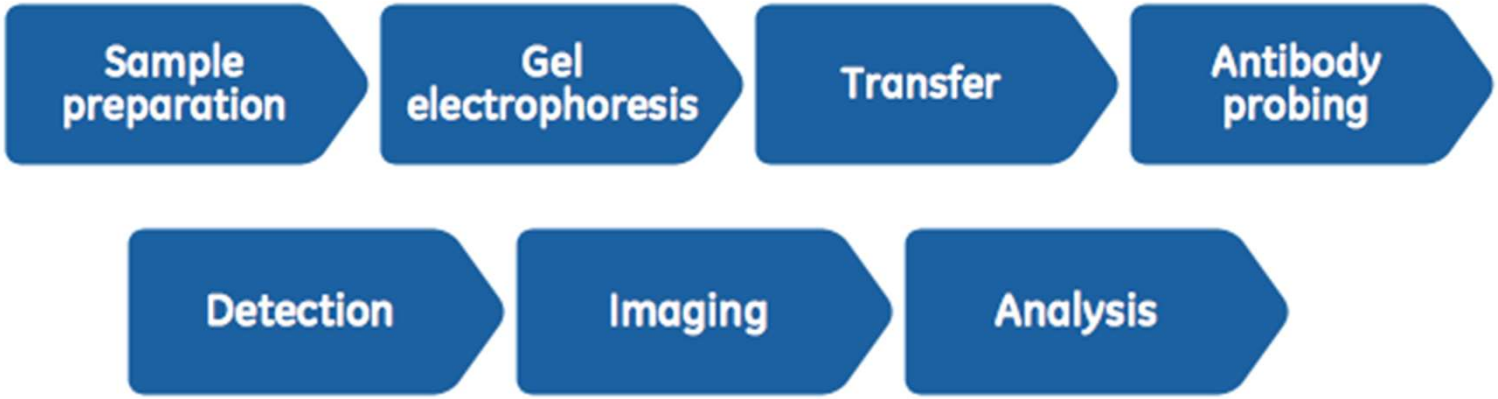
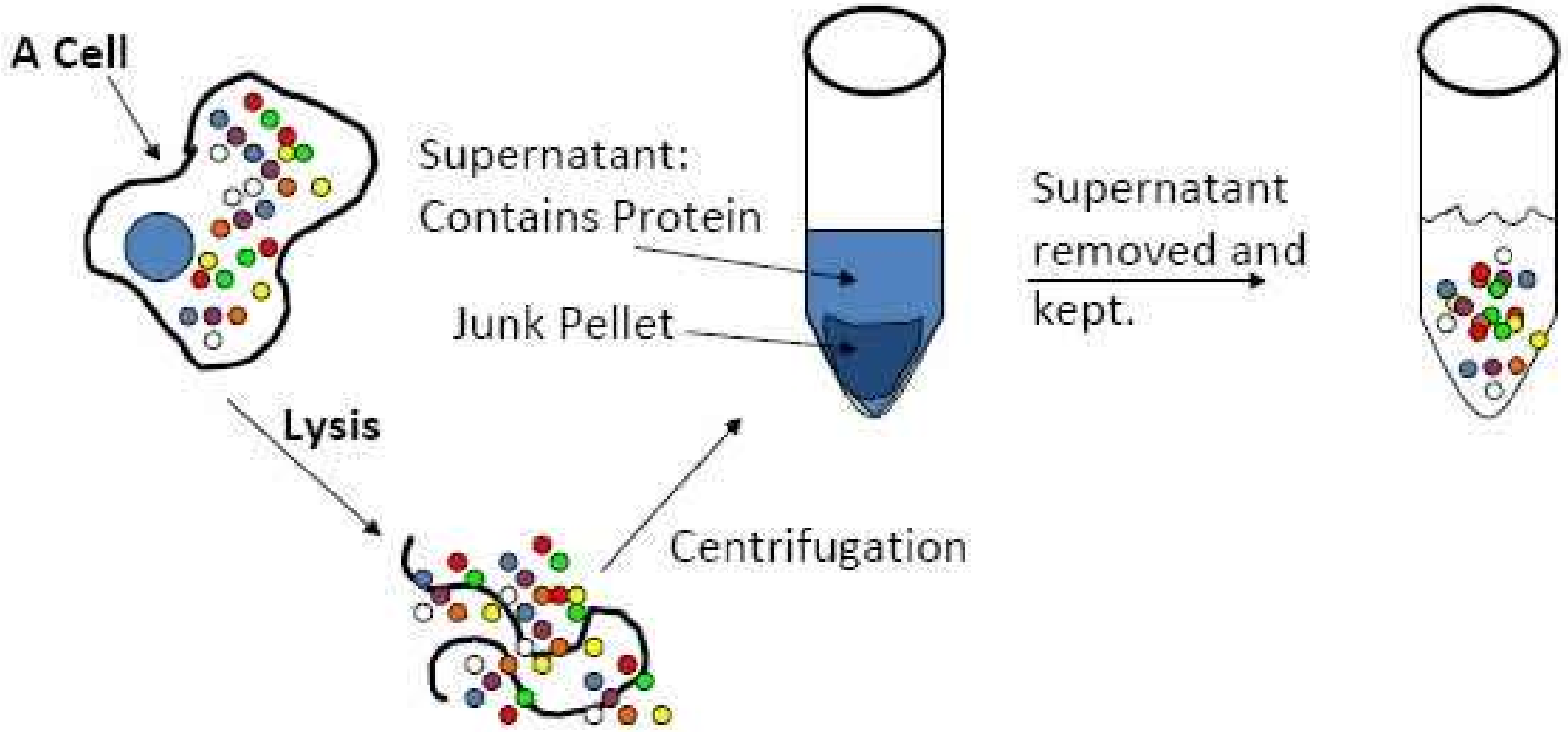


Figure 1. Standard steps in Western blotting

Sample preparation



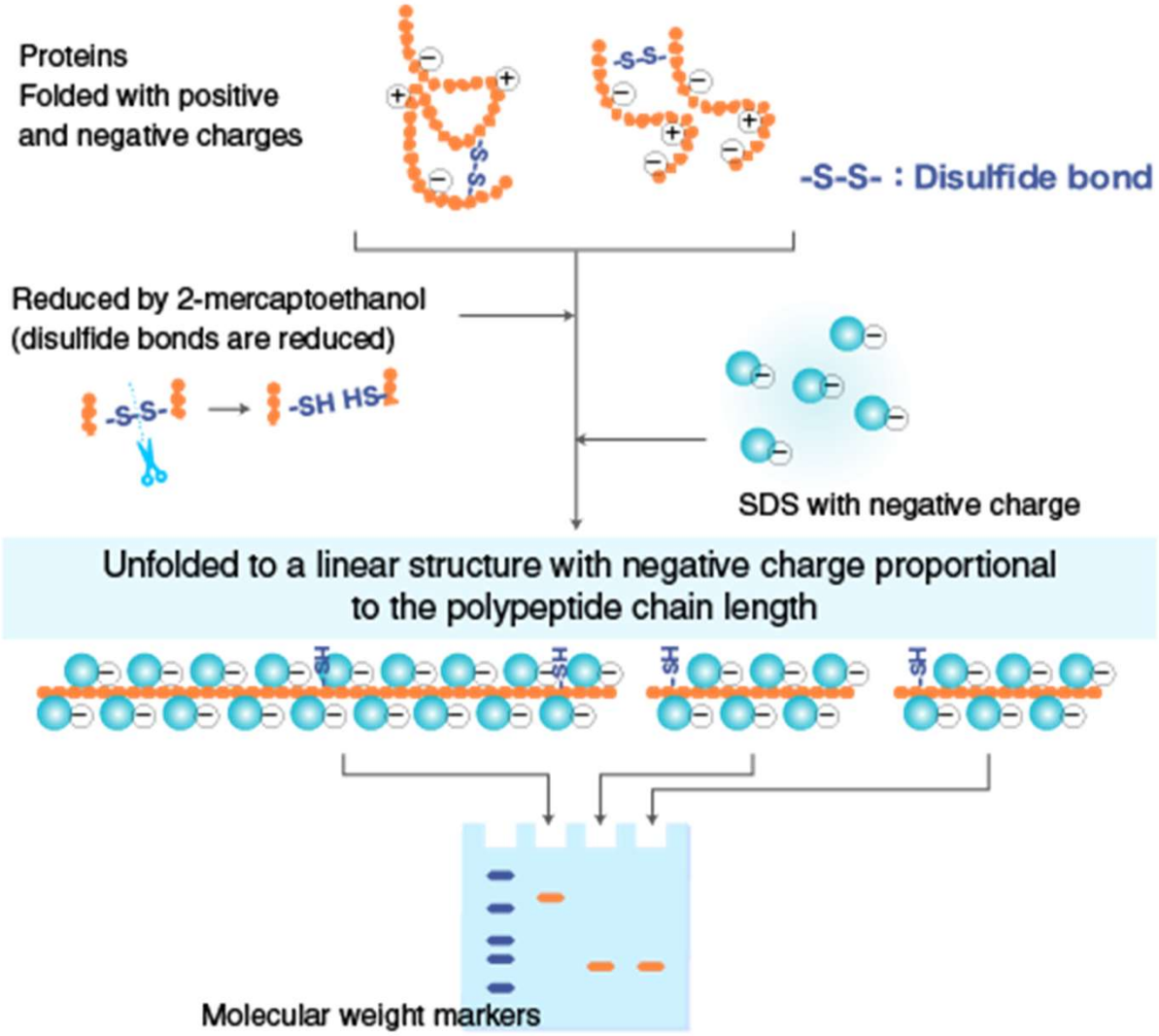
https://www.novusbio.com/support/support-by-application/western-blot/illustrated-assay.html 6/14

Figure 2. General sample preparation procedure in western blotting

Gel electrophoresis

SDS-PAGE
(SDS- Polyacrylamide gel electrophoresis)

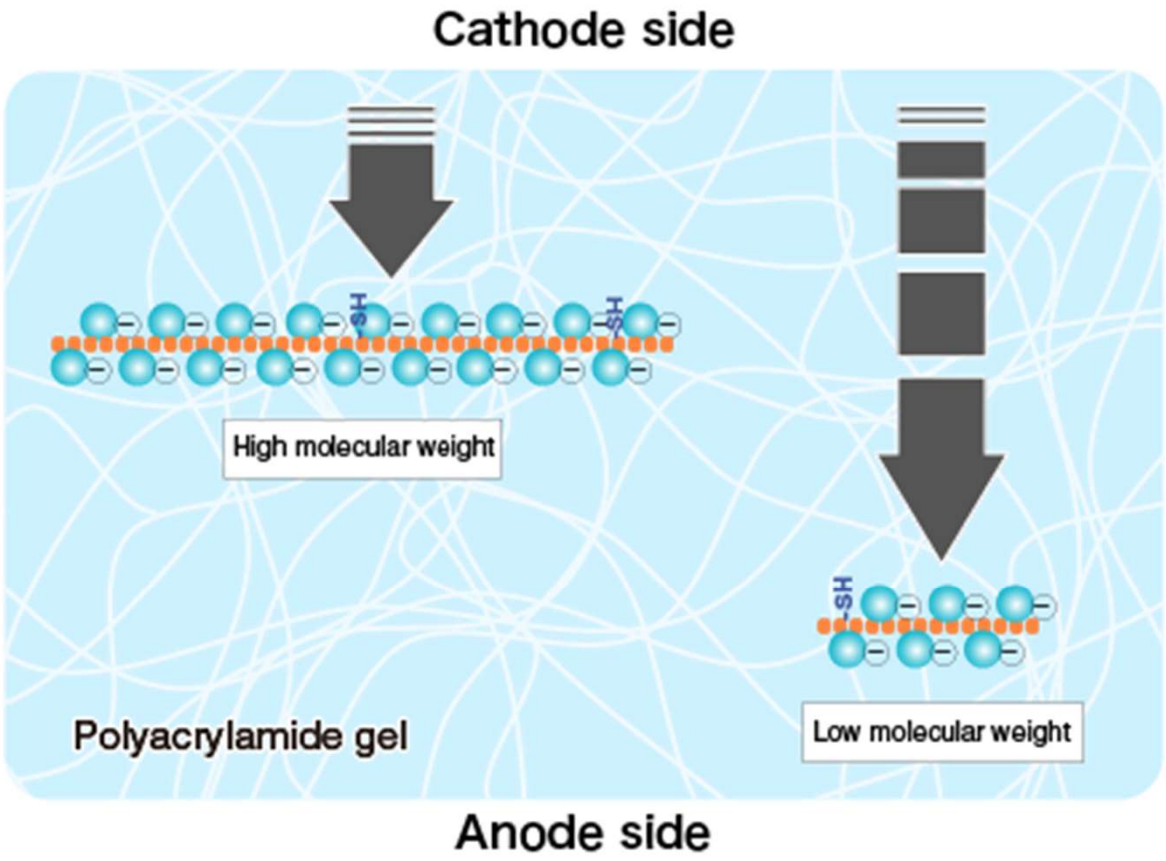
- Polyacrylamide gel
- Buffers loaded with sodium dodecyl sulfate (SDS)



https://www.mblintl.com/products/sds-polyacrylamide-gel-electrophoresis-mbli/ 6/14

Gel electrophoresis

Proteins are separated based on their polypeptide chain length by electrophoresis in a polyacrylamide gel with an appropriate mesh size.



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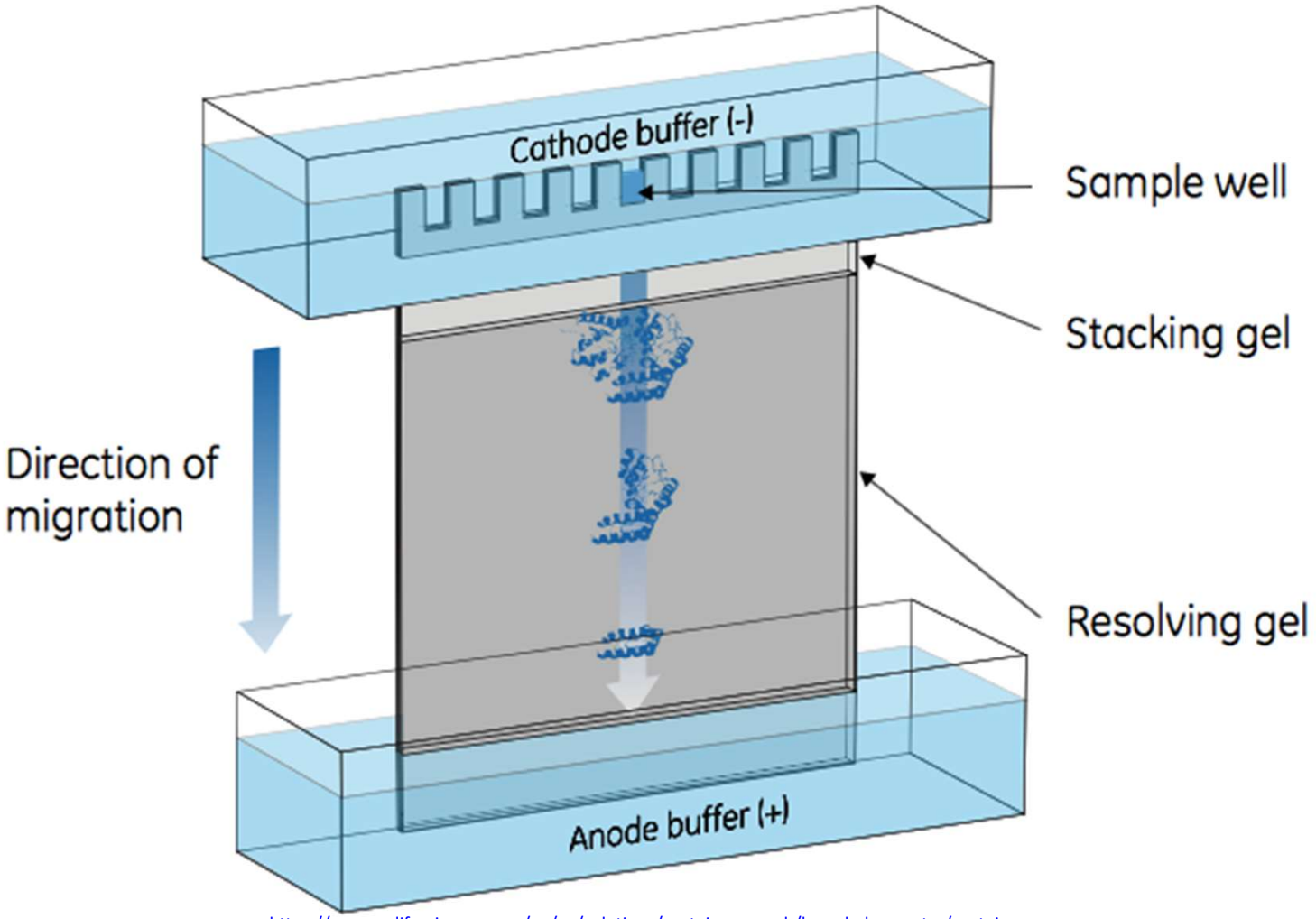
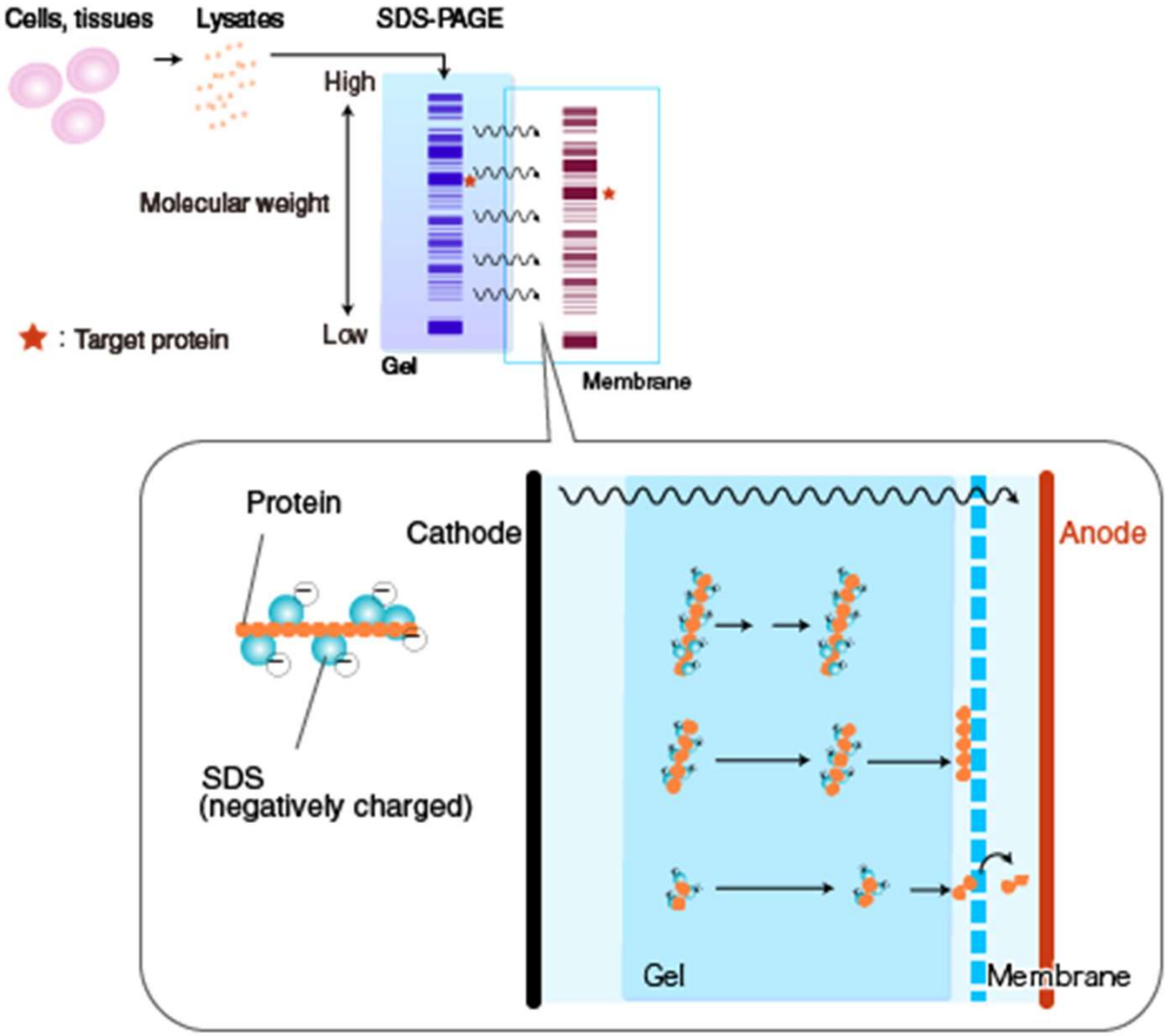
Gel electrophoresis



https://www.gelifesciences.com/en/ae/solutions/protein-research/knowledge-center/protein-purification-methods 6/14

Transfer

Proteins are separated by electrophoresis and transferred to a membrane.



https://www.mblbio.com/bio/g/support/method/westernblotting.html

Transfer

Post-transfer

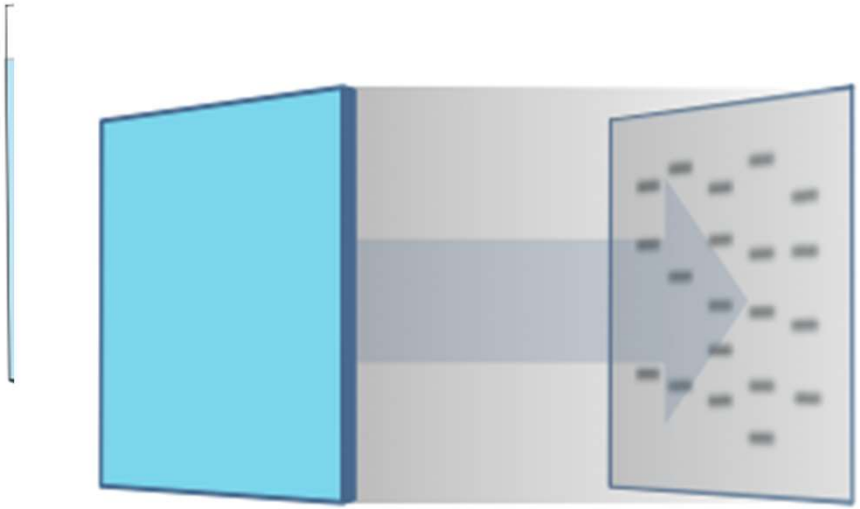
Transfer setup

Gel: No protein bands remain after effective electrotransfer

Membrane with copy of band pattern from gel

https://m.blog.naver.com/PostView.nhn?blogId=hswdb&logNo=220674682374&proxyReferer=https%3A%2F%2Fwww.google.co.jp%2F 6/17

Cathode (-)



Electrophoretic transfer

Transfer

Two very important things for transferring:

- Close contact of gel and membrane to ensure a clear image
- The placement of the membrane between the gel and the positive electrode

Two conditions:

- Wet
- Semi-dry

Two types of membrane:

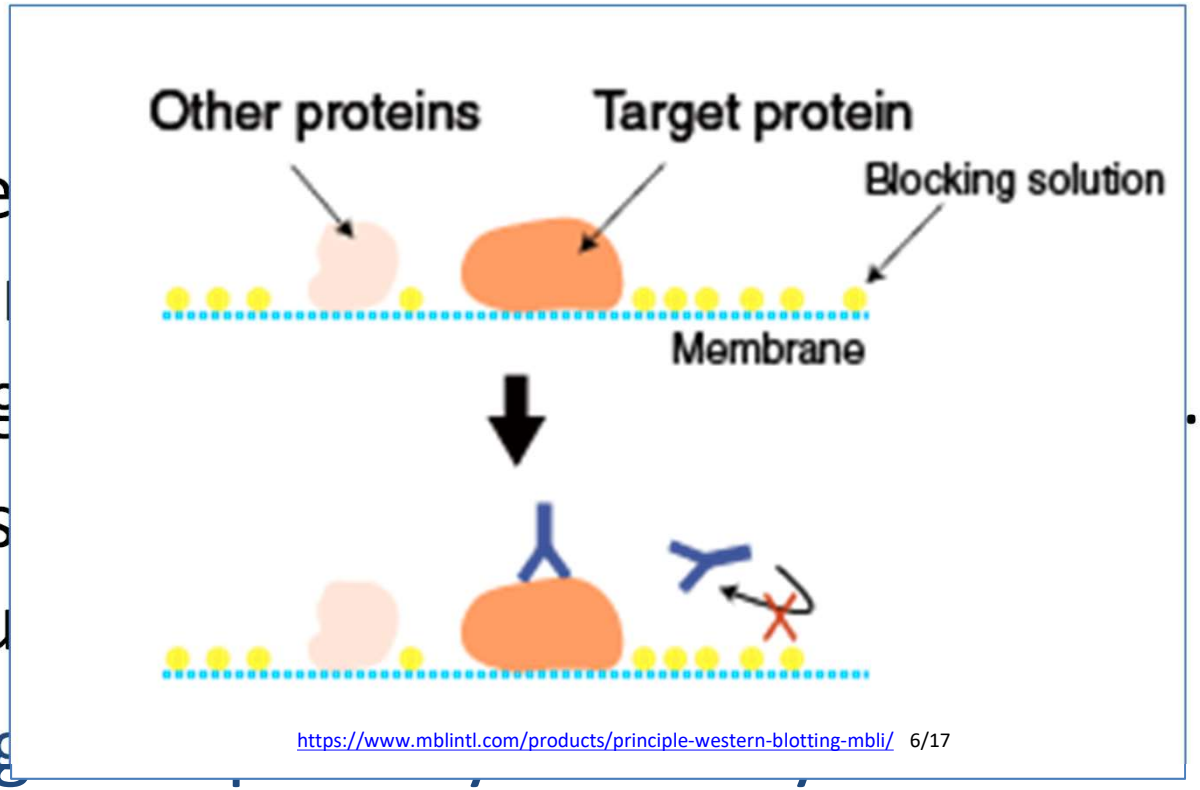
- Nitrocellulose
- PVDF (polyvinylidene difluoride)

Antibody probing

1. Blocking

A substance
sites on the
from binding

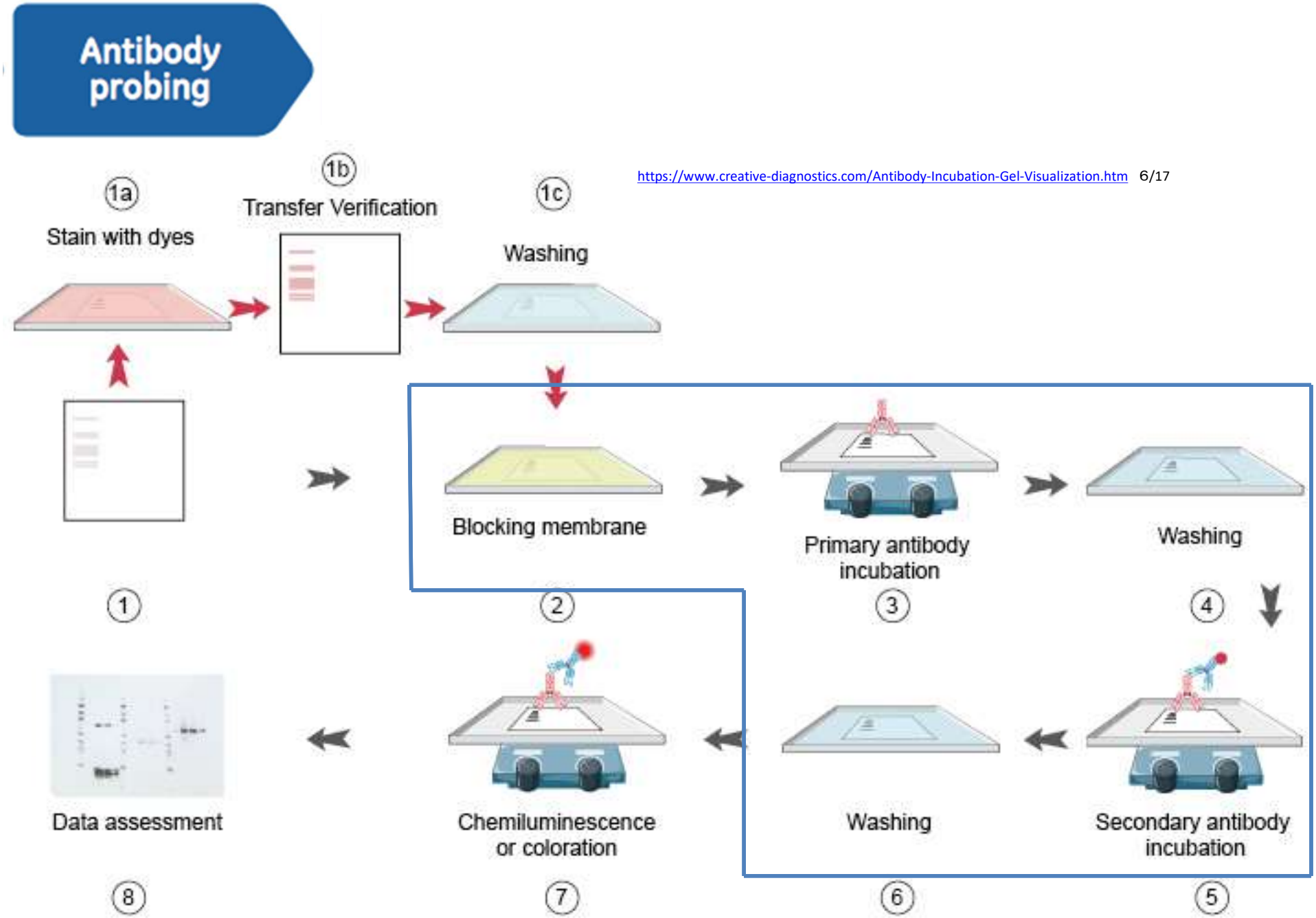
Example of s
(Bovine Seru



https://www.mblintl.com/products/principle-western-blotting-mbli/ 6/17

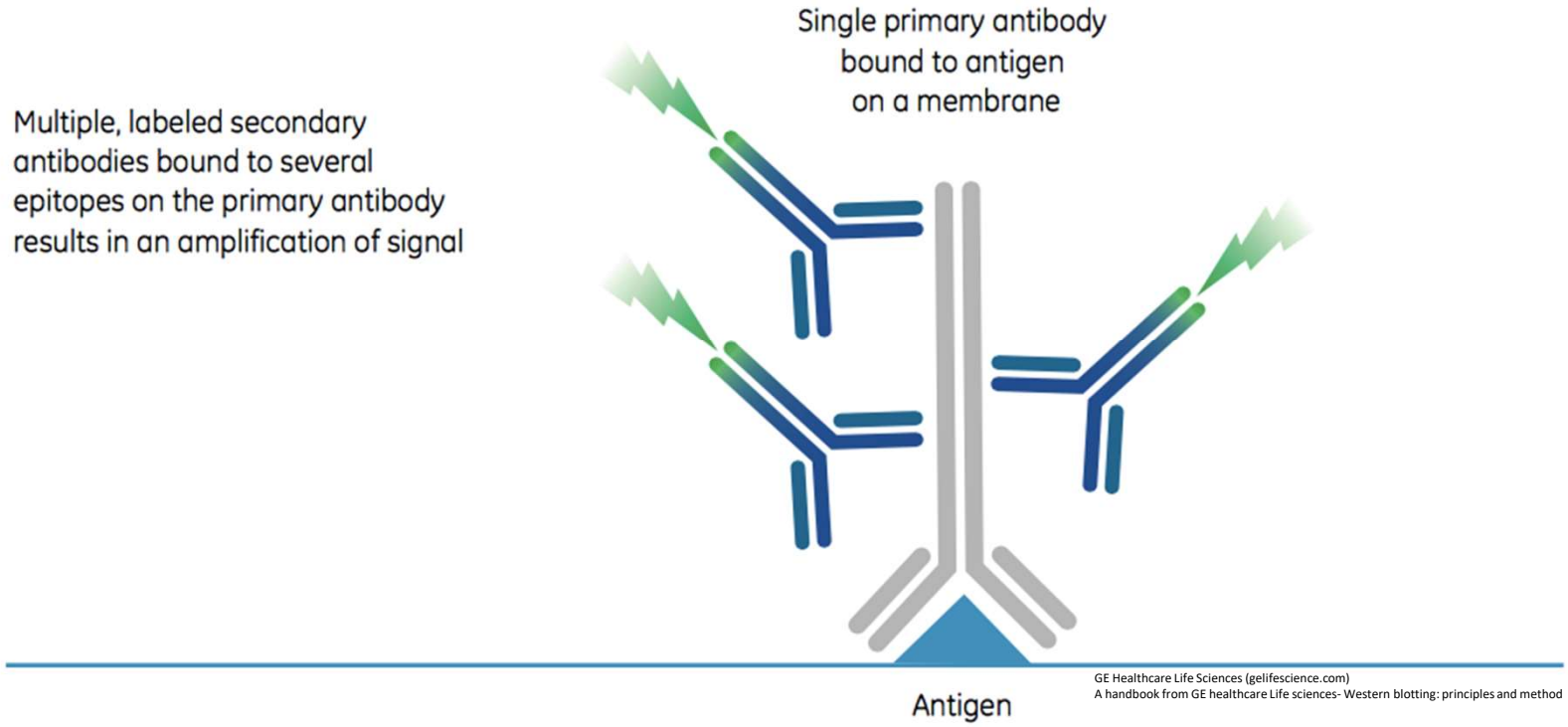
2. Incubating

3. Incubating with the secondary antibody that binds to the primary antibody.


Antibody probing
1a
Stain with dyes
1b
Transfer Verification
1c
Washing
Blocking membrane
Primary antibody incubation
Washing
Secondary antibody incubation
Washing
Chemiluminescence or coloration
Data assessment
1
2
3
4
5
6
7
8

Antibody probing

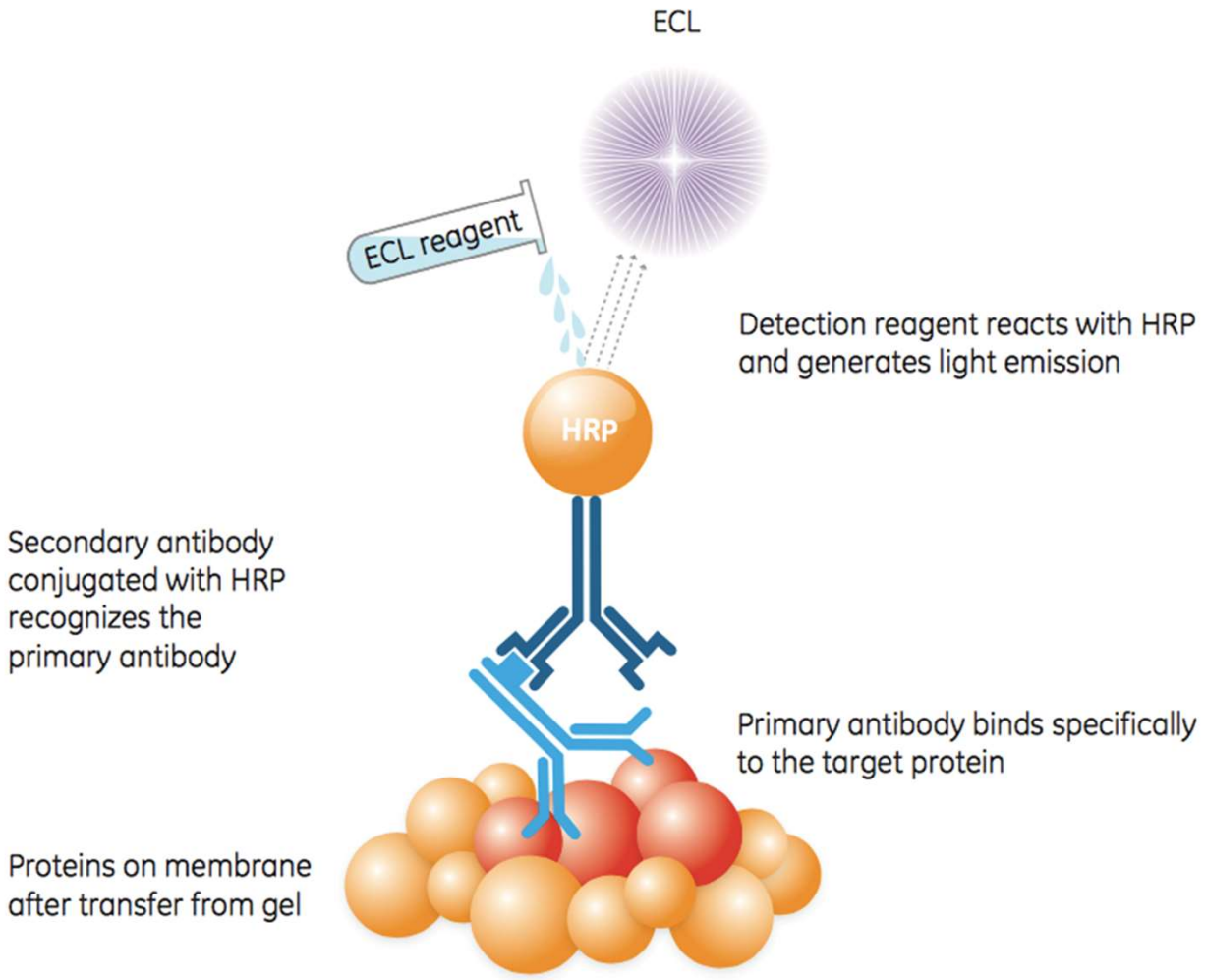
Multiple, labeled secondary antibodies bound to several epitopes on the primary antibody results in an amplification of signal



GE Healthcare Life Sciences (gelifescience.com)
A handbook from GE healthcare Life sciences- Western blotting: principles and method

The secondary antibodies can amplify the emitted signal, as many secondary antibodies can bind to the primary antibody simultaneously.

Detection



https://www.gelifesciences.com/en/gb/Solutions/Protein-Research/Knowledge-center/Western-blotting/Choosing-a-molecular-imaging-solution-part-2 6/17

Imaging

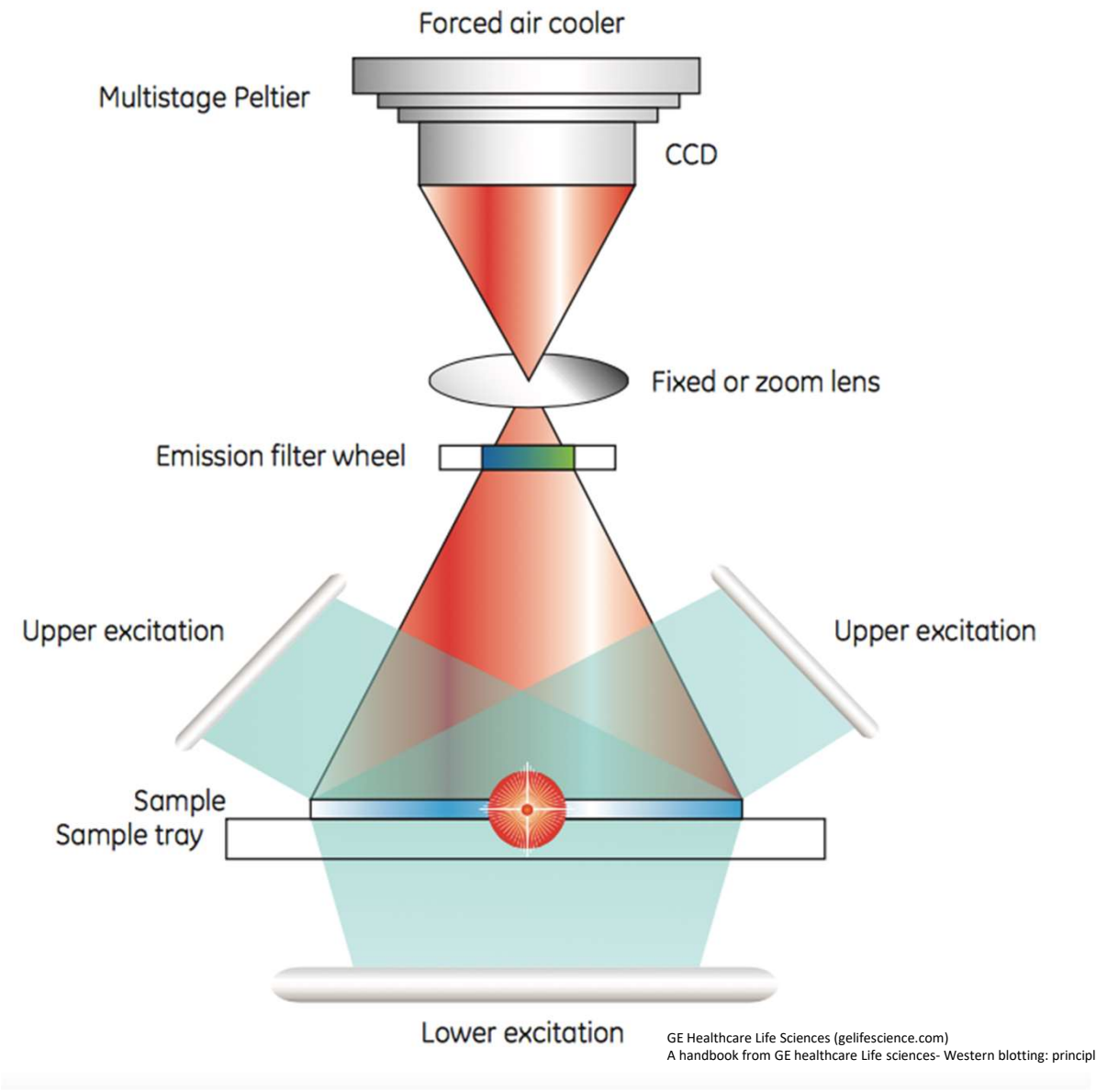


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Figure 4. Components of a typical CCD camera imaging device

Analysis

In a qualitative analysis:

- The presence of a protein of interest is confirmed.
- The amount is approximated by visual inspection.
- The size is determined by comparison with a marker.

References

- https://www.proteinatlas.org/learn/method/western+blot
- Western blotting principles and methods – a free handbook provided by GE healthcare
- The article describes Western Blotting technique, theory, and trouble shooting:
  Mahmood, T., & Yang, P.-C. (2012). Western blot: technique, theory, and trouble shooting. North American Journal of Medical Sciences, 4(9), 429-34.
  DOI:10.4103/1947-2714.100998. PubMed: 23050259


Thank You