Assiut University

Protein immuno-blotting, methods and analysis

By

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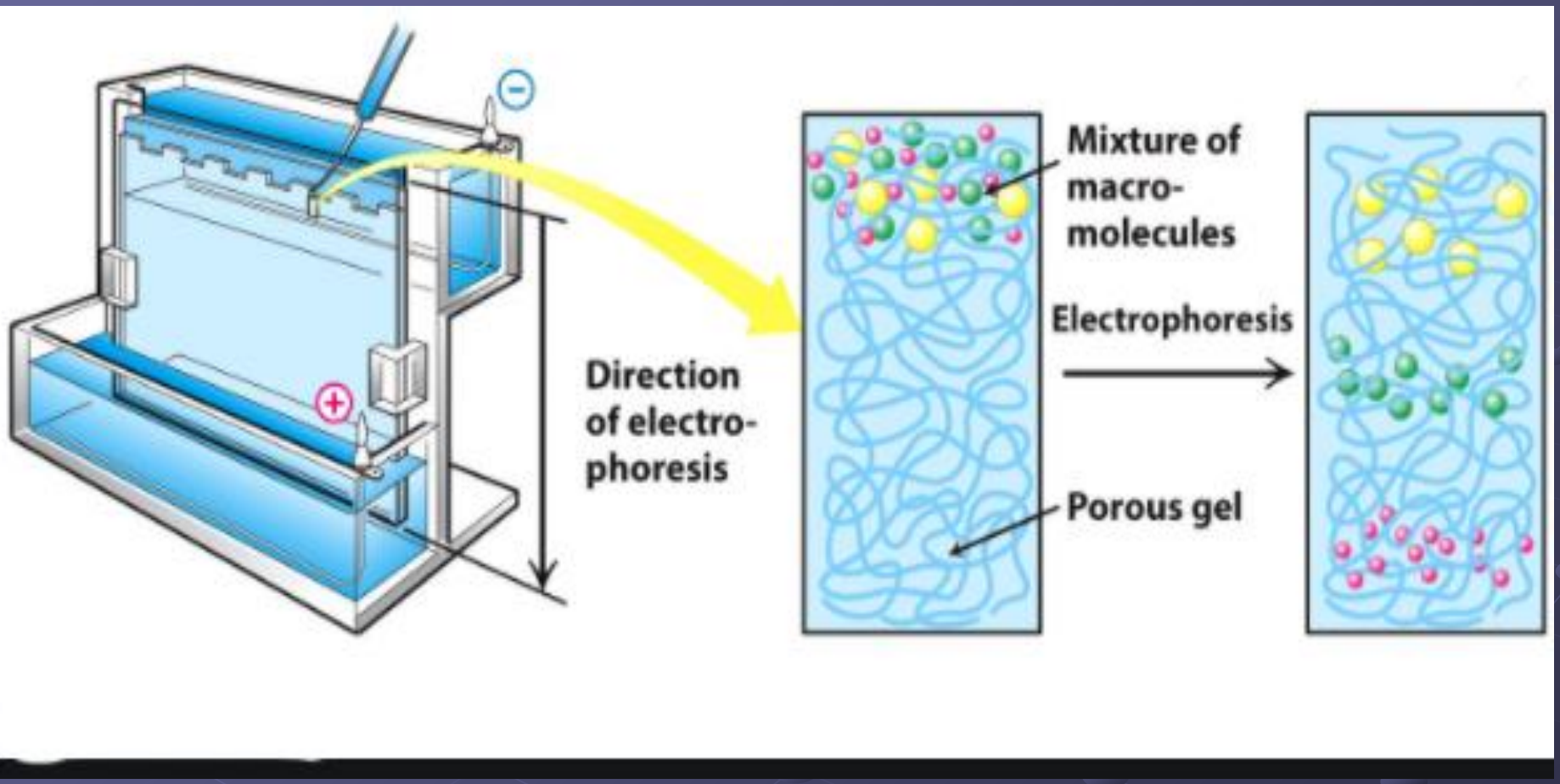
Electrophoresis

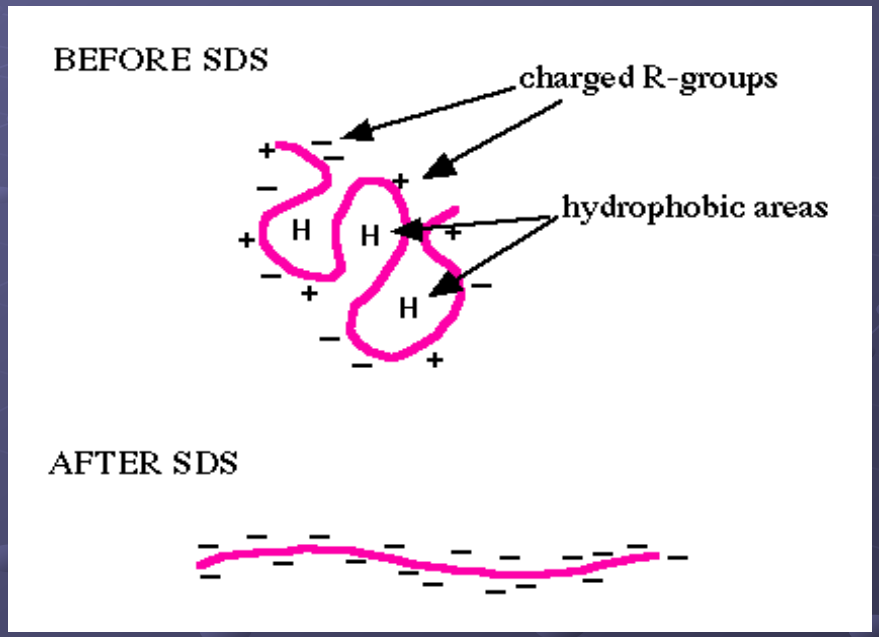
- **Cation** = positively charged ion, it moves toward the cathode (-)
- **Anion** = negatively charged ion, it moves toward the anode (+)
- **Amphoteric substance** = can have a positive/negative/zero charge, it depends on conditions

- Principle:
- Some substances have different net charges and can be separated into several fractions in external electric field.
- But velocity of a particle also depends on the:
- **size, shape of the particle and given applied voltage**


Direction of electro-phoresis
Mixture of macro-molecules
Electrophoresis
Porous gel

SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis (Laemmli 1970)

SDS (sodium dodecyl sulfate) is a detergent (soap) that can dissolve hydrophobic molecules but also has a negative charge



•Therefore, if a cell is incubated with SDS, the membranes will be dissolved, all the proteins will be solubalized by the detergent and all the proteins will be covered with many negative charges.

Protein gel (SDS-PAGE) stained with Coomassie Blue.

Western blot

It is a jock?????

Sir prof. Edwin Southern

Inventor of southern blot

Terminologies..

- The **Western blot** (alternatively, **protein immunoblot**) is an analytical technique used to detect specific proteins in a given sample of tissue homogenate or extract.
- A **Southern blot** is a method routinely used in molecular biology for detection of a specific DNA sequence in DNA samples.
- The **northern blot** is a technique used in molecular biology research to study gene expression by detection of RNA.
- **Southwestern blotting**, based along the lines of Southern blotting (which was created by Edwin Southern) and first described by B. Bowen and colleagues in 1980, is a lab technique which involves identifying and characterizing DNA-binding proteins (proteins that bind to DNA).

Western Blotting (WB)

WB is a protein detection technique that combines the separation power of SDS PAGE together with high recognition specificity of antibodies

An antibody against the target protein could be purified from serum of animals (mice, rabbits, goats) immunized with this protein

Alternatively, if protein contains a commonly used tag or epitope, an antibody against the tag/epitope could be purchase from a commercial source (e.g. anti-6 His antibody)

WB: 4 steps

1. Separation of proteins using SDS PAGE

A-sample lysis by lysis buffer (including proteases inhibitors)

B- mix with loading buffer and boil (makes the proteins solution heavy and colored)

C- SDS PAGE

2. Transfer of the proteins from the gel onto a nitrocellulose or PVDF membrane (blotting)

3. Immune reactions

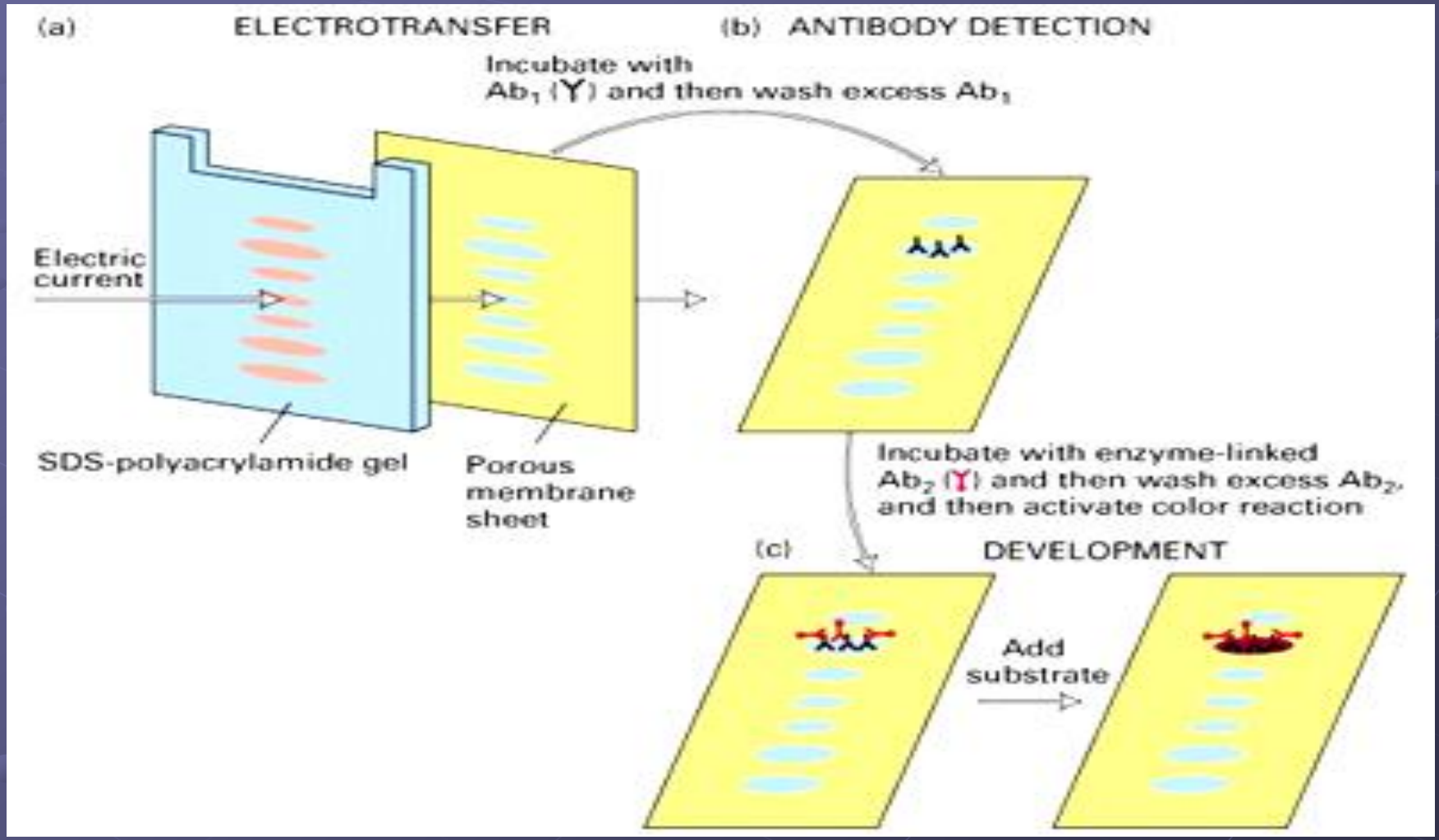
A- blocking the active sites of the membrane by blocking buffer

B- First Ab and then second Ab with enzyme linked

4. Visualization

A- Chemoluminescence method by ECL or colored method by DAB

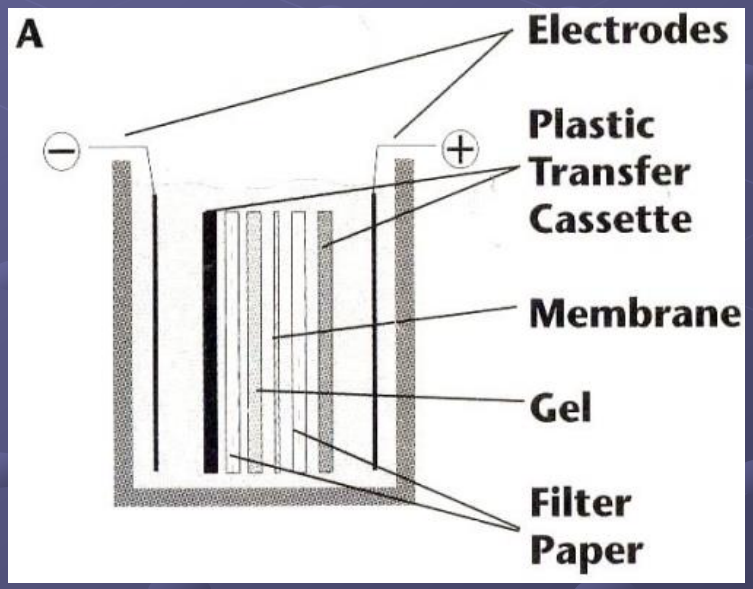
The essence of Western-blot

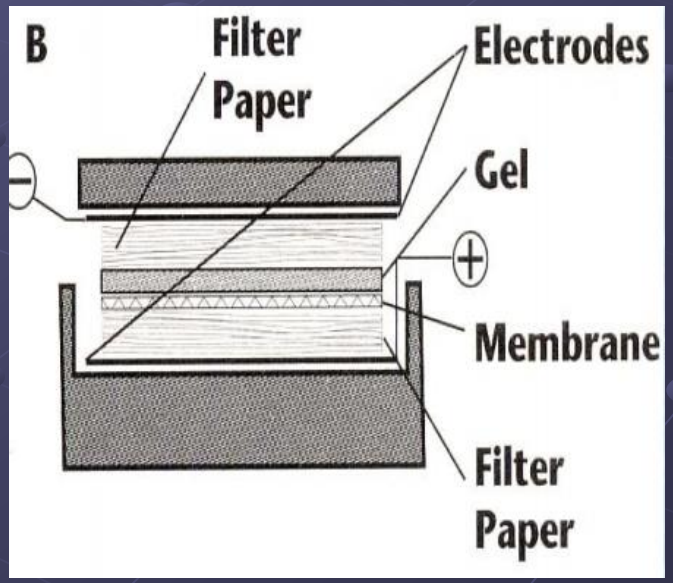


Transfer

Wet

Semi-dry

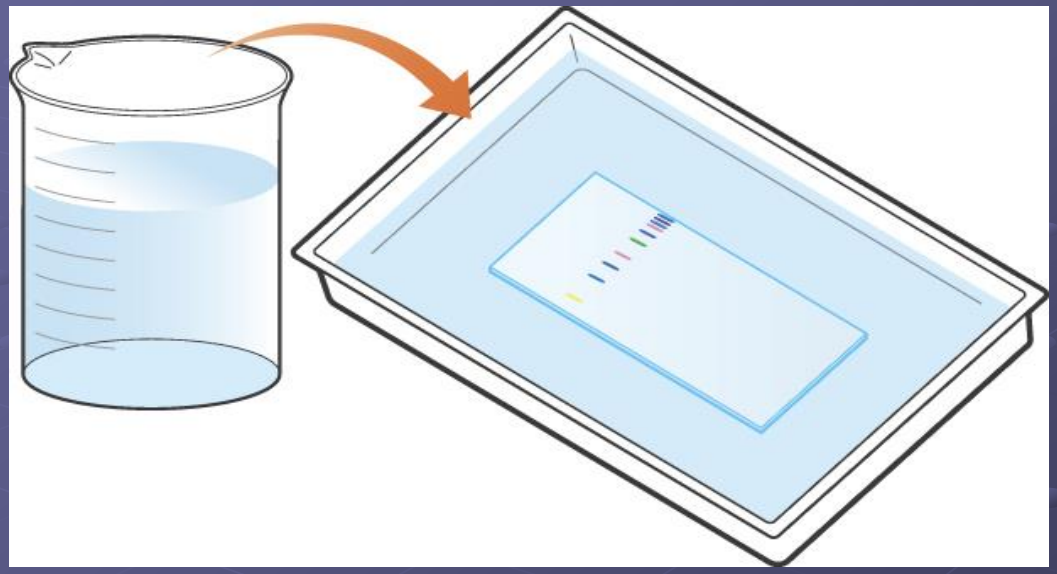




Types of membranes

Nitrocellulose (NC) high binding capacity, works well with both protein and DNA
not need methanol to preparation.
Polyvinylidene difluoride (PVDF) high capacity and stable, need methanol for preparation.
These both membranes bind proteins non-covalently.

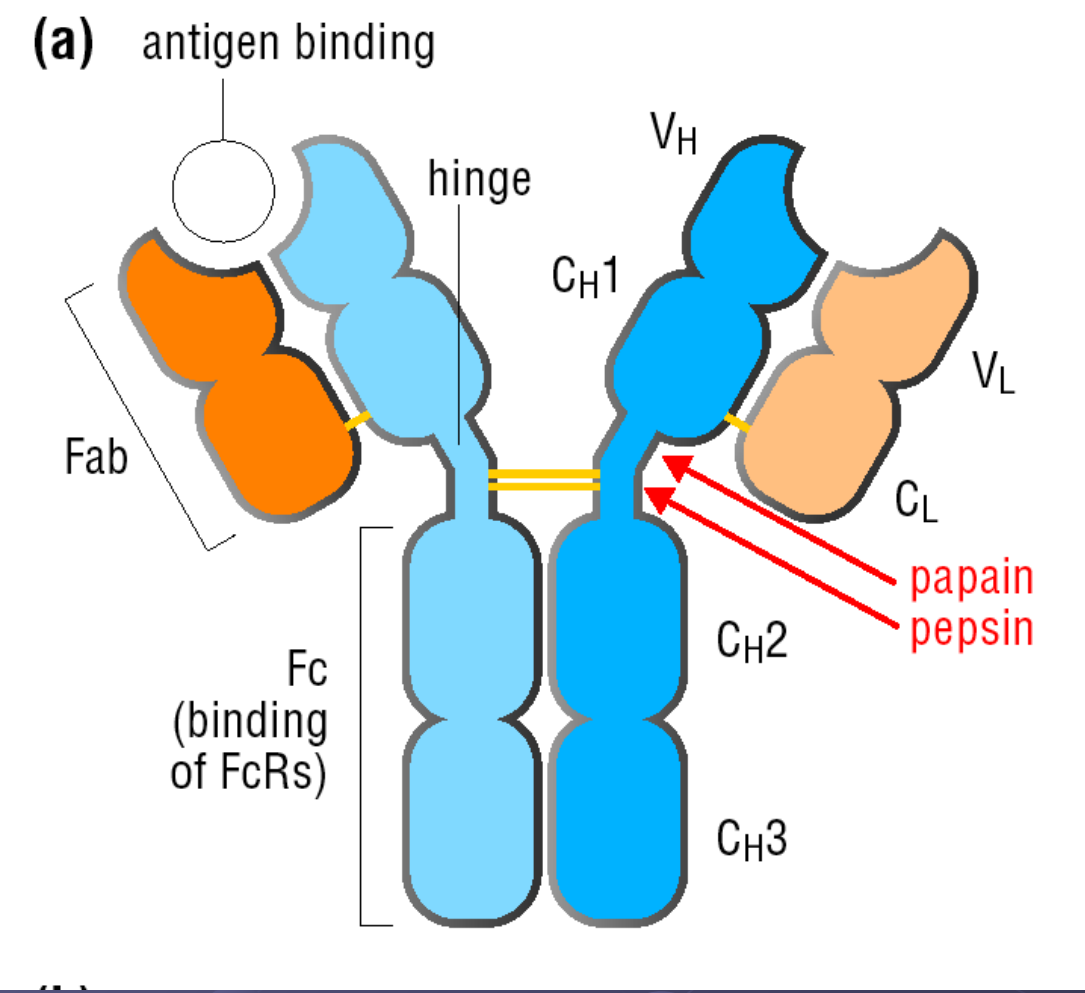
Blocking



5% non-fat milk or BSA with Tween 20: Prevents the primary antibody from binding randomly to the membrane

After blocking apply your first Ab at the specific concentration, learn how…? Wash carefully, apply secondary Ab HRB conjugated, wash carefully, detect your specific protein by detection reagent.

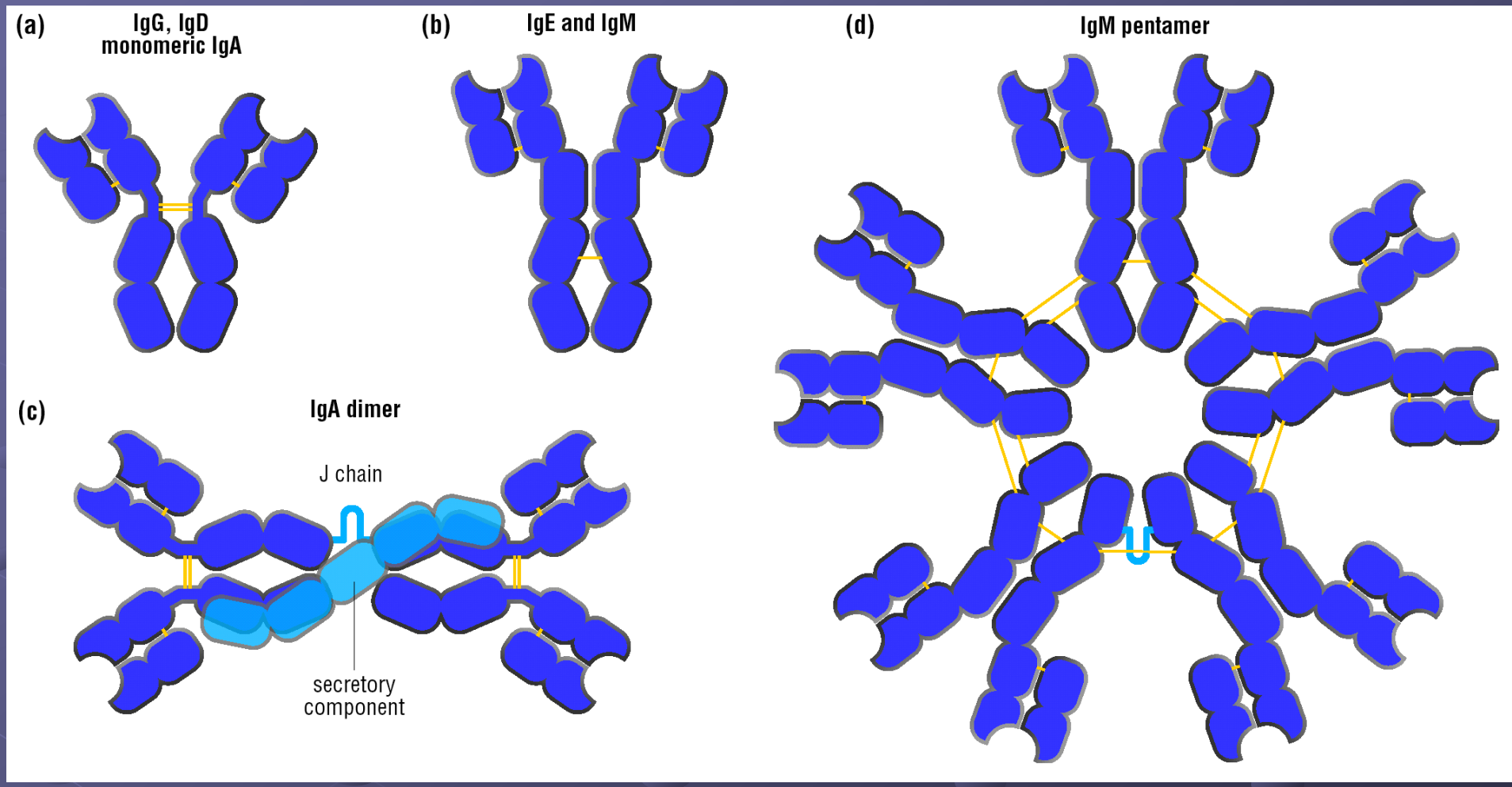
Antibody Structure



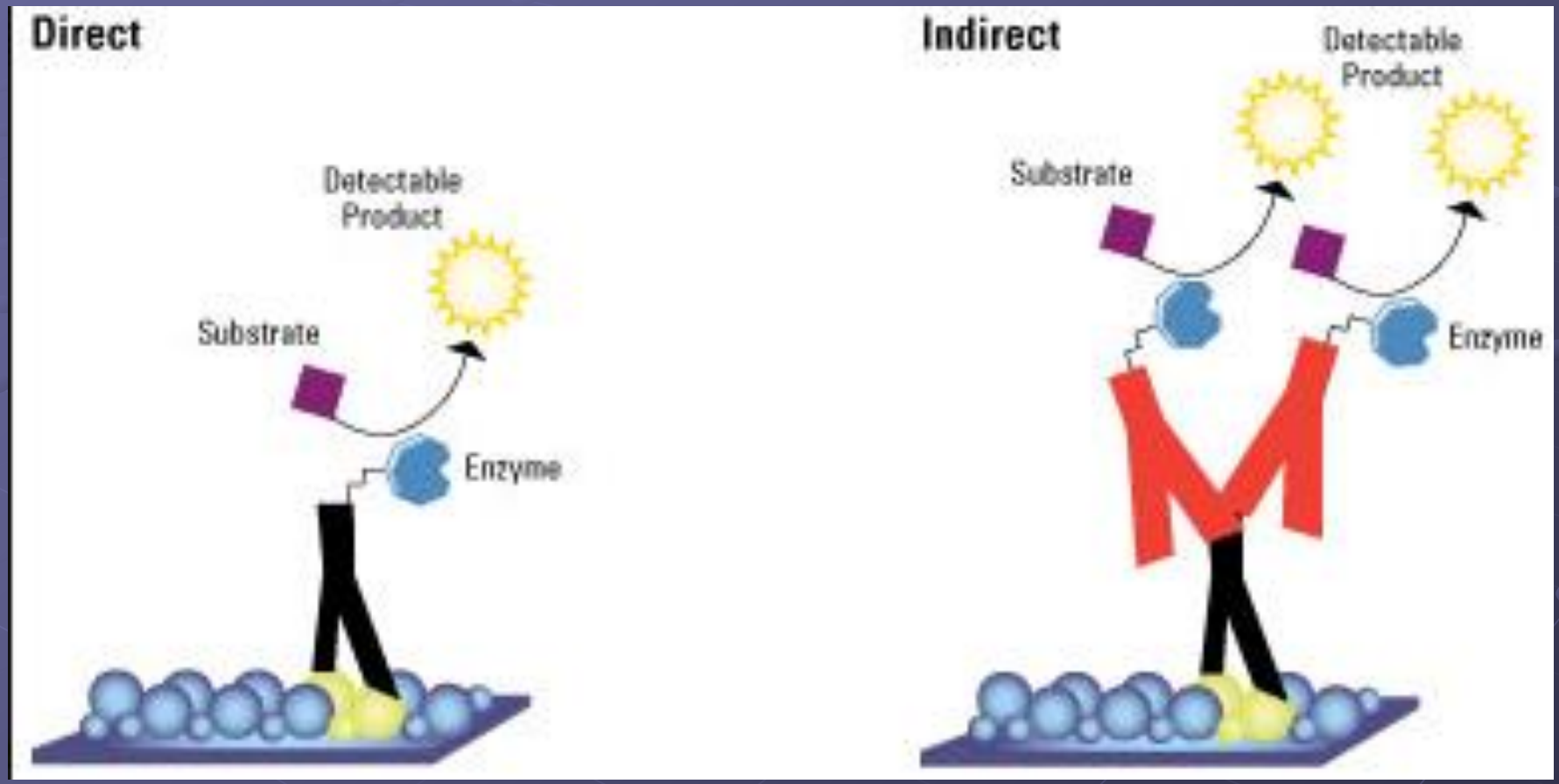
Ig domain: 110 amino acids; globular domain used in many proteins.
Variable domains, Constant domains, Hinge.
Fab: fragment antigen binding
Fc: fragment crystallizable (effector functions)

Immunoglobulins (Ig) are glycoproteins made up of **light (L)** and **heavy(H)** polypeptide chains. The simplest antibody molecule has a Y shape and consists of four polypeptide chains:two H chains and two L chains. The four chains are linked by disulfide bonds.

Antibody Classes: Structure



WB, Steps 3-4: Immuno-detection and Visualization



visualization of western blot

Most common methods

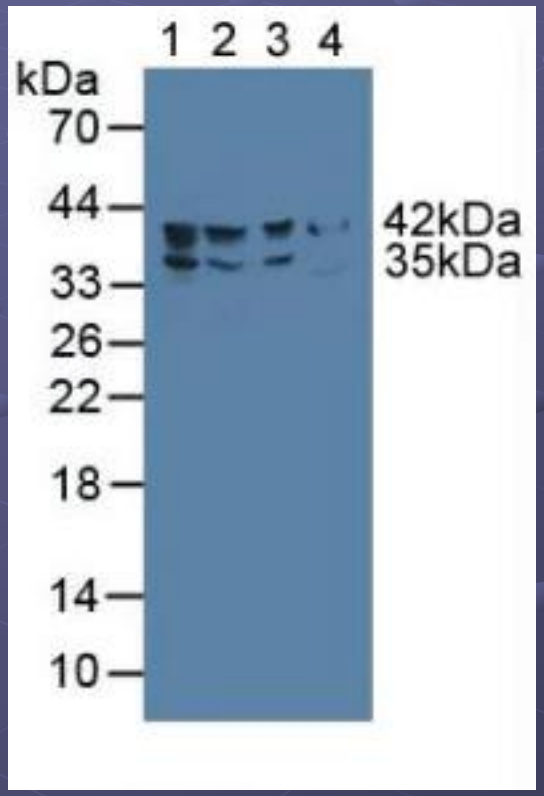
1- colorimetric, by substrate (ex. DAB) that affected by atomic O resulted from H2O2 hydrolysis by HRB enzyme linked to secondary antibody and a colour develops

2- ECL, a reagents that affected by atomic O and give luminescence that filmed on X ray films in a dark room, more sensitive than colorimetric method

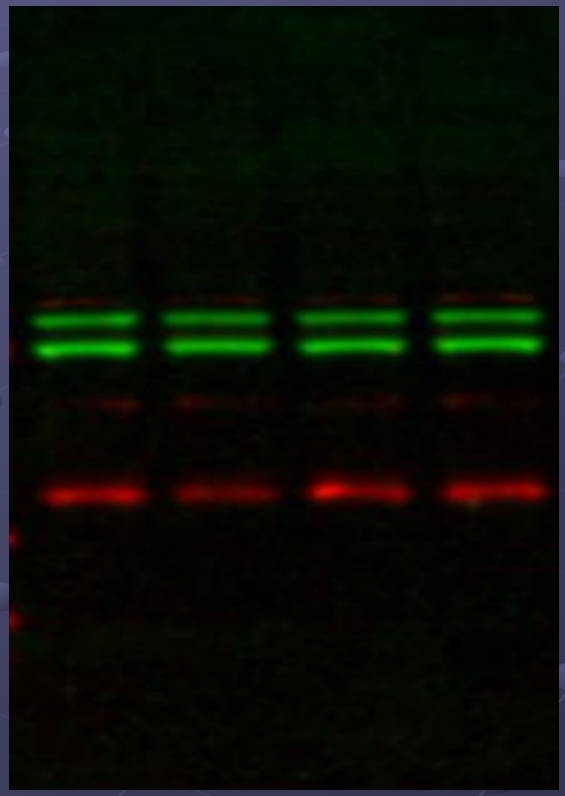
3- Fluorescent, Using a fluorescently labeled secondary antibody instead of HRP

Visualization of reactive bands

DAB



ECL

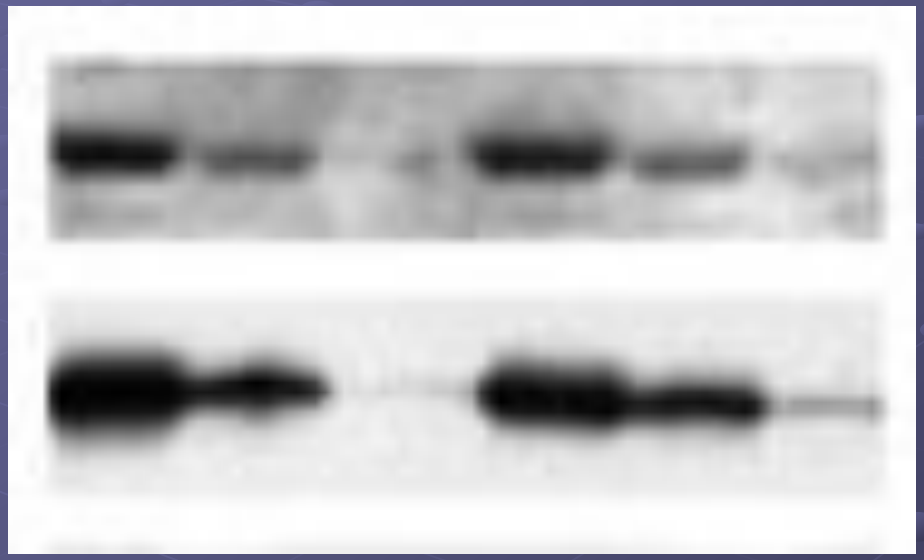


FLUOR.

Comparison between ECL and DAB detection methods

DAB

ECL

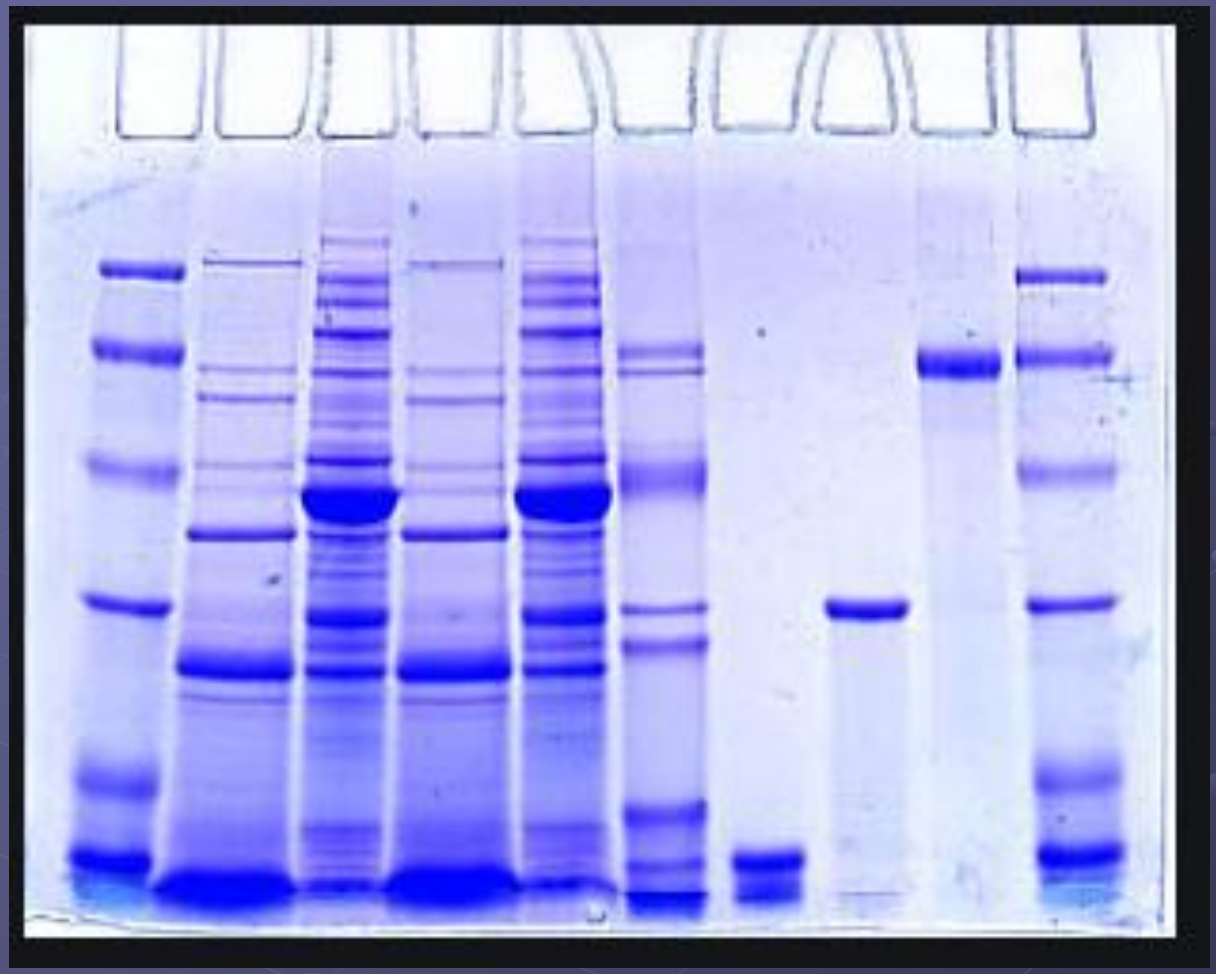


ECL is more sensitive 3-5 folds

Some points for consideration

Stacking gel

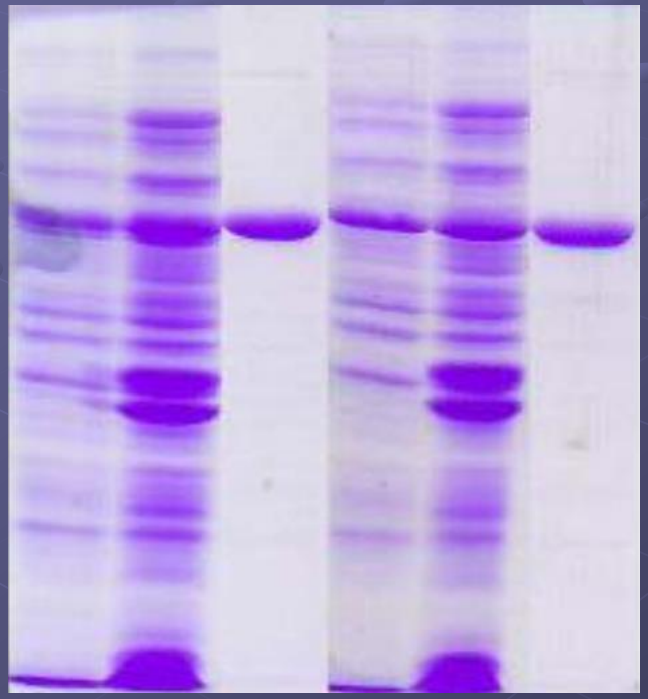
Resolving or separating gel



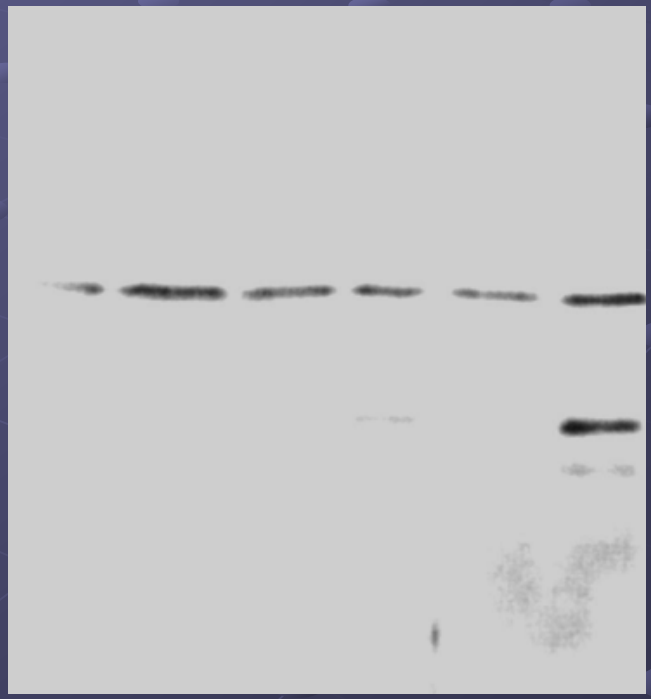
Each band does not represent a single protein, but may by a lot of proteins.

The proteins appear in the gel after staining do not represent all proteins in your sample because of the dye threshold

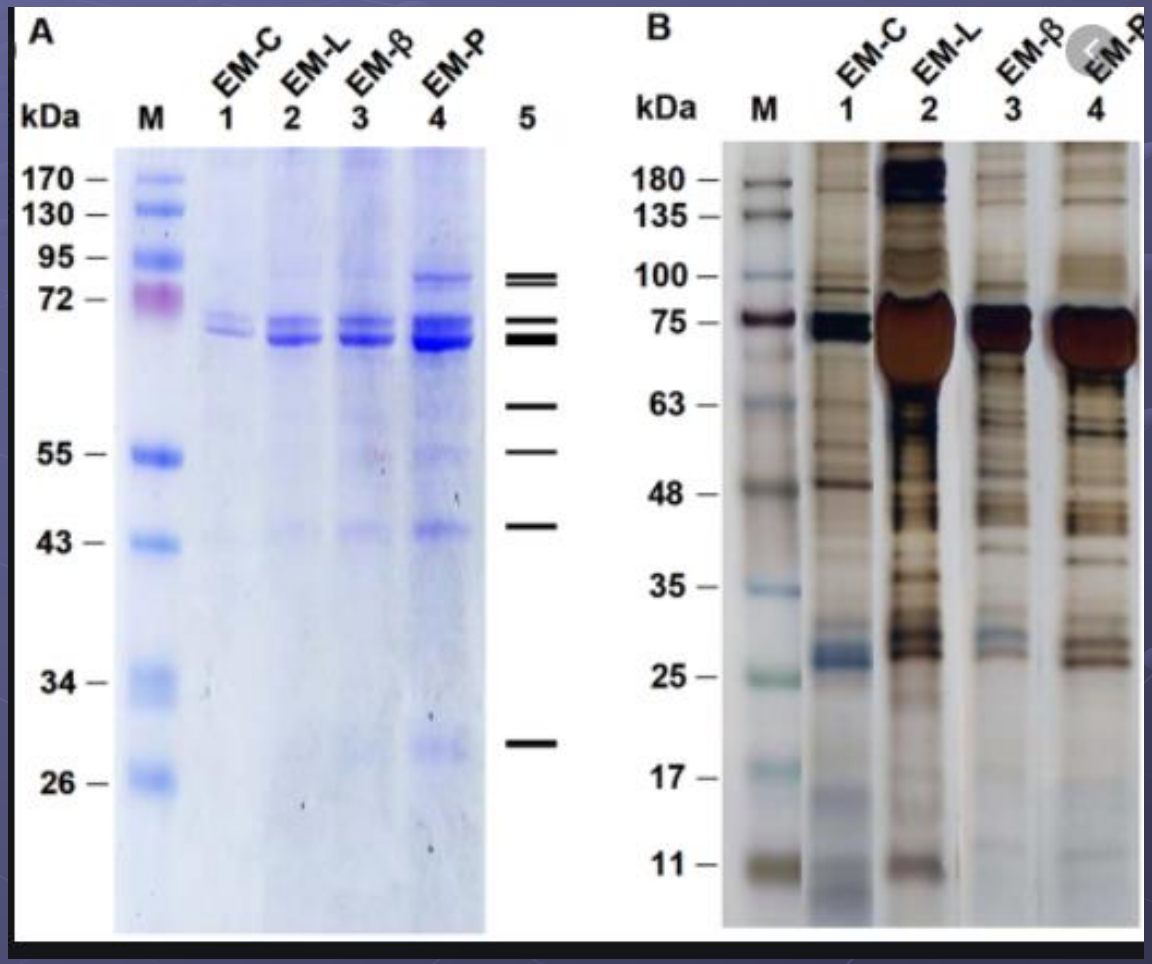
SDS page stained with coomassie blue



The same but after Western blot

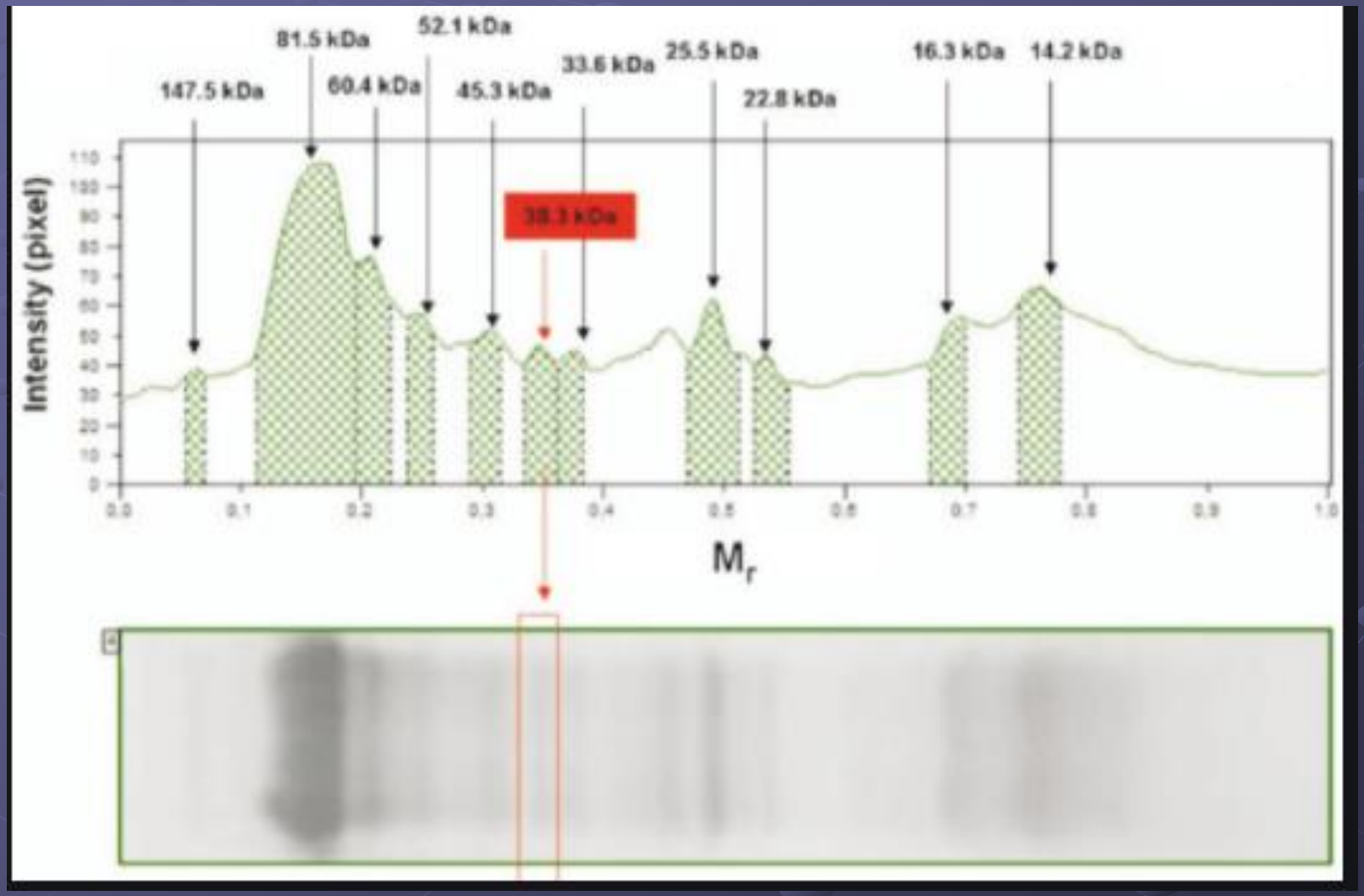


Coomassei blue vs silver stains



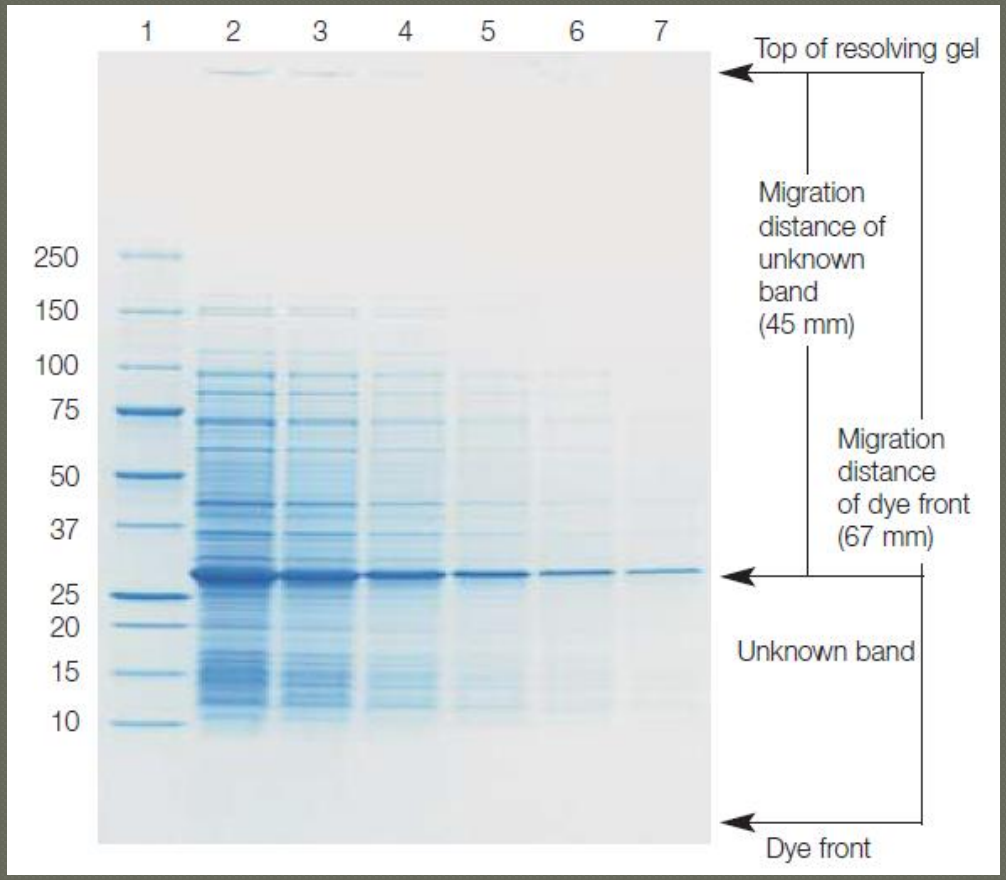
Different threshold

Protein bands analysis software

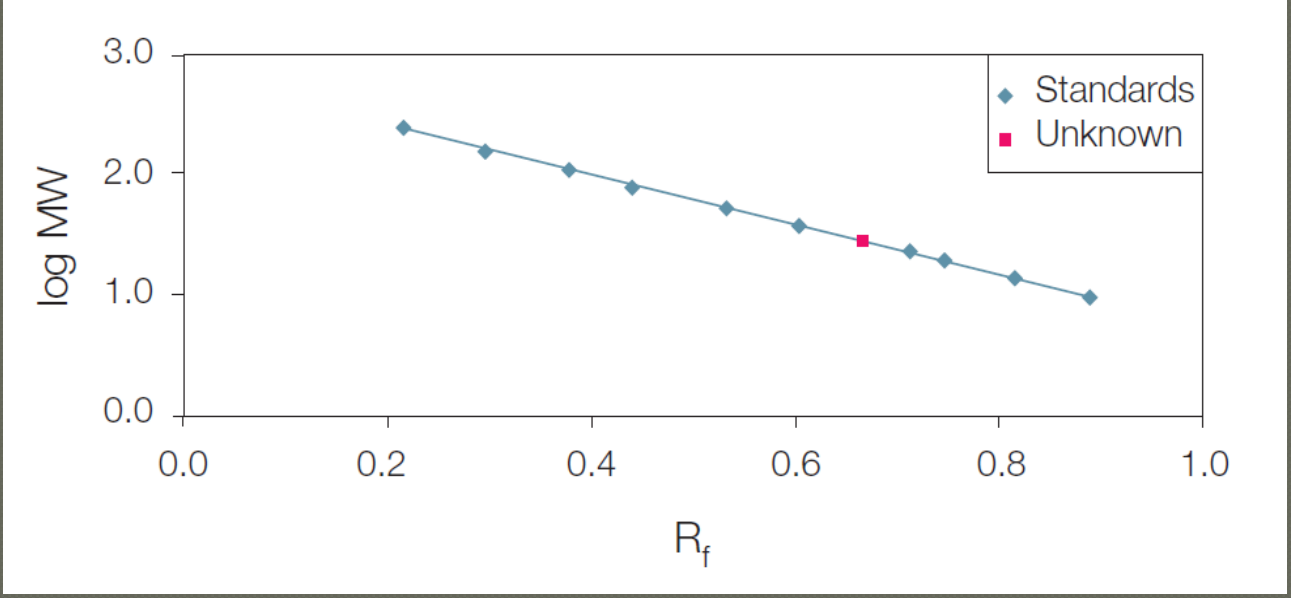


Protein molecular mass calculation

- Apparent mole. Mass from SDS gel



Determine migration distance of each standard (s)
Determine migration distance of dye front (c)
Determine the Rf (relative migration of each standard) s/c
Draw a plot between log mol weight of standards (y) and Rf (X)
Migration distance of unknown protein: 45 mm
Migration distance of dye front: 67 mm
So Rf = 45 mm/67 mm = 0.67
Determine it in the curve and obtain the corresponding log Mol. W

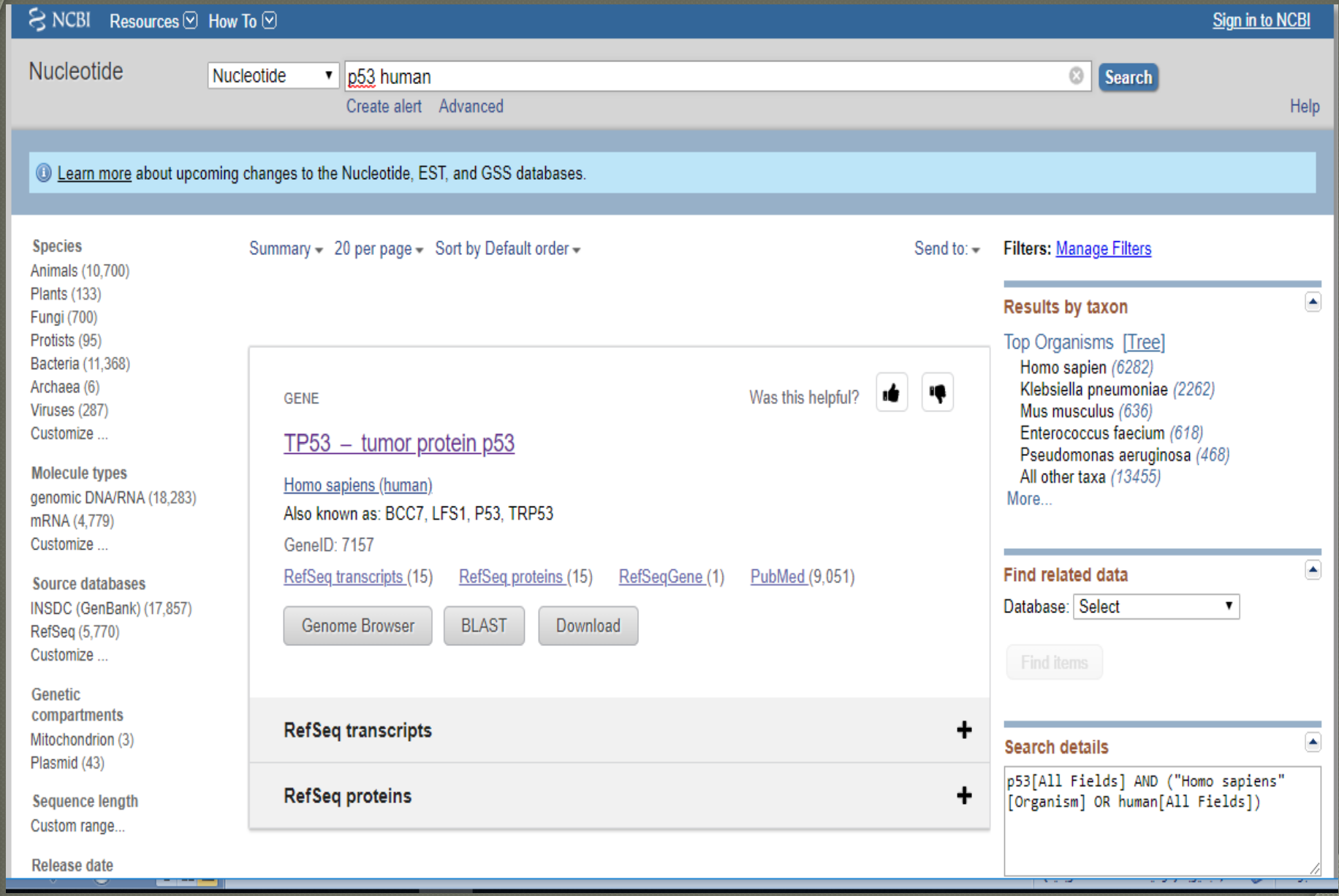


- Exact mol. Weight calculated from its amino acids residues

M.W. of protein = # amino acids x 110 Da

Or use mol. Weight calculator

- Mol weight of protein from its gDNA get cDNA from NCBI, and divide it by 3 to get number of amino acids and multiply by 110 to get the mol. Weight in Dalton


NCBI Resources How To
Sign in to NCBI
Nucleotide
Nucleotide
p53 human
Search
Create alert Advanced
Help
Learn more about upcoming changes to the Nucleotide, EST, and GSS databases.
Species
Animals (10,700)
Plants (133)
Fungi (700)
Protists (95)
Bacteria (11,368)
Archaea (6)
Viruses (287)
Customize ...
Molecule types
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mRNA (4,779)
Customize ...
Source databases
INSDC (GenBank) (17,857)
RefSeq (5,770)
Customize ...
Genetic compartments
Mitochondrion (3)
Plasmid (43)
Sequence length
Custom range...
Release date
Summary
20 per page
Sort by Default order
Send to:
Filters: Manage Filters
GENE
Was this helpful?
TP53 – tumor protein p53
Homo sapiens (human)
Also known as: BCC7, LFS1, P53, TRP53
GeneID: 7157
RefSeq transcripts (15)
RefSeq proteins (15)
RefSeqGene (1)
PubMed (9,051)
Genome Browser
BLAST
Download
RefSeq transcripts
RefSeq proteins
Results by taxon
Top Organisms [Tree]
Homo sapien (6282)
Klebsiella pneumoniae (2262)
Mus musculus (636)
Enterococcus faecium (618)
Pseudomonas aeruginosa (468)
All other taxa (13455)
More...
Find related data
Database: Select
Find items
Search details
p53[All Fields] AND ("Homo sapiens"[Organism] OR human[All Fields])

https://www.ncbi.nlm.nih.gov/nuccore/?term=p53+human

Search

See more...

Items: 1 to 20 of 23721

<< First < Prev Page 1 of 1187 Next > Last >>

Found 23952 nucleotide sequences. Nucleotide (23721) EST (196) GSS (35)

1. **Homo sapiens** mRNA for **P53**, complete cds
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   Accession: AB082923.1 GI: 23491728
   Protein PubMed Taxonomy
   GenBank FASTA Graphics

2. **Homo sapiens p53 (p53)** gene, exon 7 and partial cds
   110 bp linear DNA
   Accession: JF923572.1 GI: 349734069
   Protein Taxonomy
   GenBank FASTA Graphics

3. **Homo sapiens p53 (p53)** gene, exon 6 and partial cds
   113 bp linear DNA
   Accession: JF923571.1 GI: 349734067
   Protein Taxonomy
   GenBank FASTA Graphics

4. **Homo sapiens p53 (p53)** gene, exon 5 and partial cds
   183 bp linear DNA
   Accession: JF923570.1 GI: 349734065
   Protein Taxonomy
   GenBank FASTA Graphics

5. **Homo sapiens p53 (p53)** gene, exon 4 and partial cds
   279 bp linear DNA

Recent activity

Turn Off Clear

- p53 human (23721) — Nucleotide
- p53 (70496) — Nucleotide
- p53 [Drosophila melanogaster] — Gene
- TP53 tumor protein p53 [Homo sapiens] — Gene
- p53 AND (alive[prop]) (13991) — Gene

See more...

https://www.ncbi.nlm.nih.gov/nuccore/AB082923.1

Homo sapiens mRNA for P53, complete cds

GenBank: AB082923.1

FASTA Graphics

Go to:

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VERSION     AB082923.1
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REFERENCE   1
  AUTHORS   Azuma,K., Shichijo,S., Maeda,Y., Nakatsura,T., Nonaka,Y., Fujii,T.,
            Koike,K. and Itoh,K.
  TITLE     Mutated p53 gene encodes a nonmutated epitope recognized by
            HLA-B*4601-restricted and tumor cell-reactive CTLs at tumor site
  JOURNAL   Cancer Res. 63 (4), 854-858 (2003)
   PUBMED   12591737
REFERENCE   2  (bases 1 to 2451)
  AUTHORS   Shichijo,S. and Itoh,K.
  TITLE     Direct Submission
  JOURNAL   Submitted (26-MAR-2002) Shigeki Shichijo, Kurume Univ. School of
            Med., Dep. Immunol.; 67-Asahi-machi, Kurume, Fukuoka 830-0011,
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Customize view

Analyze this sequence

Run BLAST

Pick Primers

Highlight Sequence Features

Find in this Sequence

Articles about the TP53 gene

RBM38 plays a tumor-suppressor role via stabilizing the p53- [J Exp Clin Cancer Res. 2018]

TP53-dependence on the effect of doxorubicin and Src inhibitor combination [Tumour Biol. 2018]

Polymorphisms of p53 promoter and susceptibility to ι [Clin Exp Obstet Gynecol. 2016]

See all...

Pathways for the TP53 gene

Ferroptosis

Mitophagy - animal

Fluid shear stress and atherosclerosis

See all...

https://www.ncbi.nlm.nih.gov/nuccore/AB082923.1

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ORIGIN
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     1561 gcccacttca ccgtactaac cagggaagct gtccctcact gttgaatttt ctctaacttc
```

variants for the TP53 gene.

More about the TP53 gene

This gene encodes a tumor suppressor protein containing transcriptional activation, DNA binding, and oligomerization domains. The encoded pr...

Also Known As: BCC7, LFS1, P53, TRP53

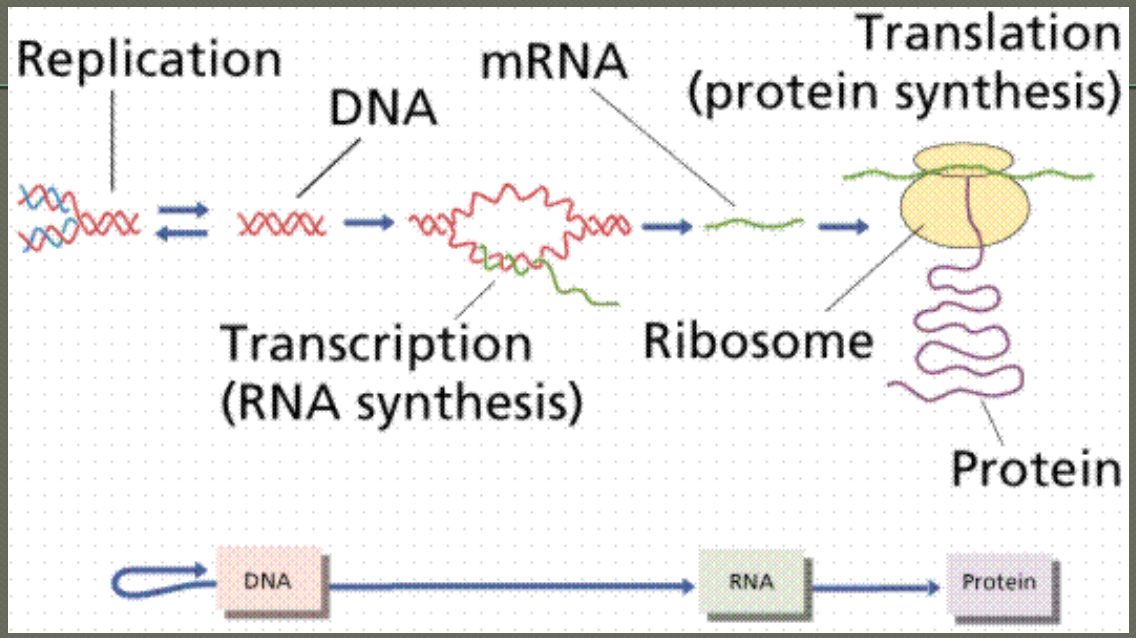
Homologs of the TP53 gene

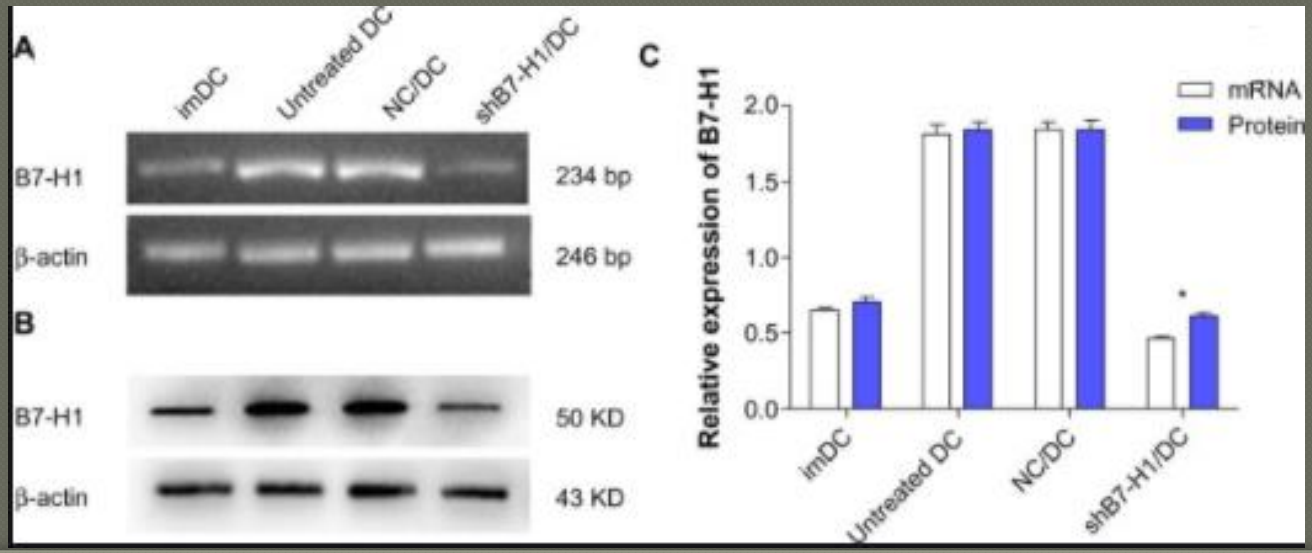
The TP53 gene is conserved in chimpanzee, Rhesus monkey, dog, cow, mouse, rat, zebrafish, and frog.

Related information

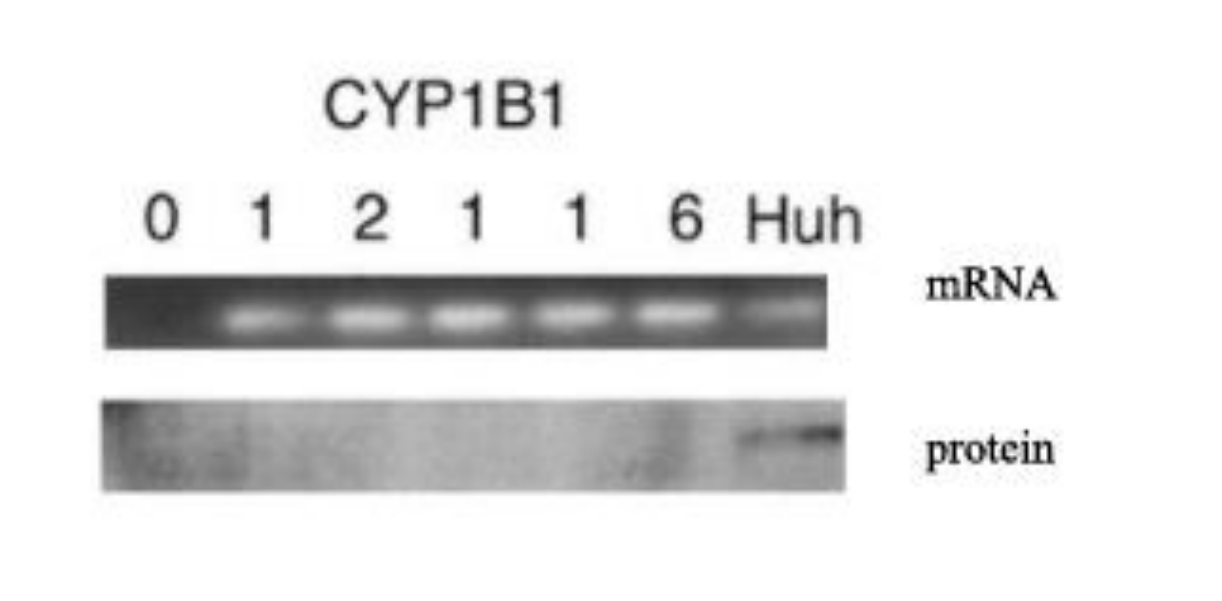
- Protein
- PubMed
- Taxonomy
- BioSystems
- Component Of
- Full text in PMC
- Functional Class
- Gene
- OMIM
- Probe
- PubMed (Weighted)

RT-PCR vs western blot

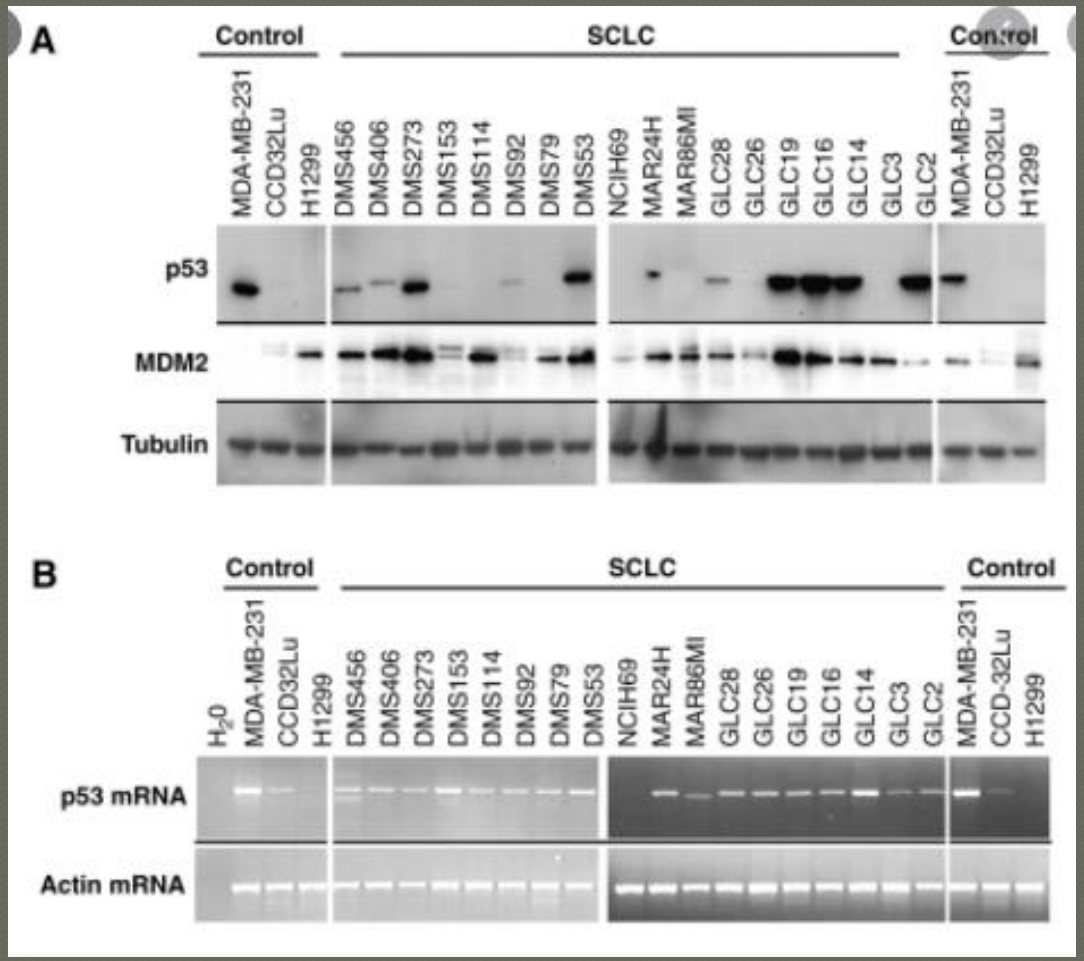




mRNA level does not always match its corresponding protein level



P53 protein and gene expression are not matching



References and additional Reading

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  An excellent introduction

- Richardson (1981) The Anatomy and Taxonomy of Protein Structure Adv. Protein Chem. 34: 167-339

  Good historical perspective

C. Branden, J. Tooze. “Introduction to Protein Structure.” Garland Science Publishing, 1999.

C. Chothia, T. Hubard, S. Brenner, H. Barns, A. Murzin. “Protein Folds in the All-β and ALL-α Classes.” Annu. Rev. Biophys. Biomol. Struct., 1997, 26:597-627.

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Thank you