

# We can raise antibodies against a specific antigen, how

[Science](#), [Biology](#)



We Can Raise Antibodies Against a Specific Antigen, How? BY loveyal 2345

Midterm 2 Review Antibodies Experimental Purpose: We can "raise"

antibodies against a specific antigen (protein of interest) How? Polyclonal: 1 antigen with many antibodies that bind to specific sites on the antigen

(Received by injecting animal with protein of interest, waiting for that animal to build antibodies (B-lymphocytes). The lymphocytes are then extracted

which give us the polyclonal antibodies. Monoclonal: 1 antibody that binds to a specific site on the antigen. (These are received by the same way as

polyclonal, except you only extract one antibody, and place that into a cancer cell to create a chimera of the two, the immortal cancer cell then acts like

the monoclonal antibody. ) These are the best to use in experiments because

they are specific to only ONE protein of interest. These antibodies can be used

in experiments to: Purify a protein of interest Visualize a particular protein in a live system or in a gel How??

Probe the gel to visualize where a protein is. Probing Protein Structure 1) X-

ray crystallography - Spend h your life producing sufficiently pure protein

and obtaining a crystal protein (Crystallizing the proteins is a hard process) "

Shoot" crystal protein with light, electrons, or radiation and examine the

diffraction patterns with extremely powerful computers -Analyze all the data

while considering the amino-acid sequence and build a 3-D model of the

protein. ) NMR-Nuclear Magnetic Resonance (Used rarely) - For small proteins

only - " Shoot" concentrated pure proteins with strong magnetic field to

generate hydrogen atom vibrations. - Use computer program to measure

reconstruct the structure of the protein by measuring the hydrogen atom

vibrations. Mass spectrometry is used as a precursor to both of these experiments. It generates the amino-acid sequence.

Protein Purification 1) Grow Cells with protein of interest (transferred on plasmid or native cell) 2) Lyse Cells -homogenization of tissues" did in lab - cell lysis buffers" break cell membrane -sonication" send sound waves through the cell to break membrane -pin-hole lysis" push mixture through an extremely tiny hole (Force large molecules through a small opening causes them to break apart) 3) Centrifugation A) Regular Centrifugation B)

Differential Centrifugation: Sequential centrifugation @ increasing speeds (low to high) -low speed pellets = big things -high speed pellets= small things C) Velocity Centrifugation layer cell and lysate over a " density gradient" and centrifuge to separate by density. Remove layers to separate proteins. D)

Equilibrium Sedimentation: another name for C 4) Column Chromatography 3 types Ion exchange (charge separation)" protein adheres to beads of an opposite charge Gel filtration (size separation)" matrix has holes, the large proteins come out last Affinity (Affinity separation)" beads have something on it that only your protein binds to. ) Electrophoresis (small volume separation or detection) -use polyacrylimide gel (creates a " mesh" in the gel to separate proteins by size and charge. separates denatured proteins 6) Isoelectric focusing based on isoelectric point of protein" 2D electrophoresis Griffiths Experiment Conclusion: heat killed bacteria transformed nonviolent bacteria Extract of heat killing S-strain transform R-strain to become S-strain Isolated " transforming material" (TM) and determined it was DNA not proteins that carried genetic information. (Took 15 years) How do we test??

Added proteases Injected into mouse Mouse should live (According to beliefs during that time period) Mouse however dies Added nucleases Mouse should die (According to beliefs during that time period) Mouse however lives

This illustrated that DNA carried the genetic information Hershey-chase Experiments Bacteriophages" virus that infect bacteria Inject DNA into bacteria (naked)" DNA unprotected by proteins Protein shell left outside of bacteria Label phages Label protein 7 groups of phages Label DNA in other groups of phages Mix both phage types with bacteria Blend bacterial mixture so that any viral parts outside the cell are ripped off Pellet bacteria and observe that only DNA label types is seen in pelleted bacteria Proved DNA carries genetic information 1) Grow bacteria with light DNA (14N) and heavy DNA (1 5N) which will separate to ifferent levels upon density-gradient centrifugation 2) Transfer heavy DNA and place in flask with light isotope Allows to eliminate conservative view 3) Heat DNA from step 2 to make it single stranded, then centrifuge.