

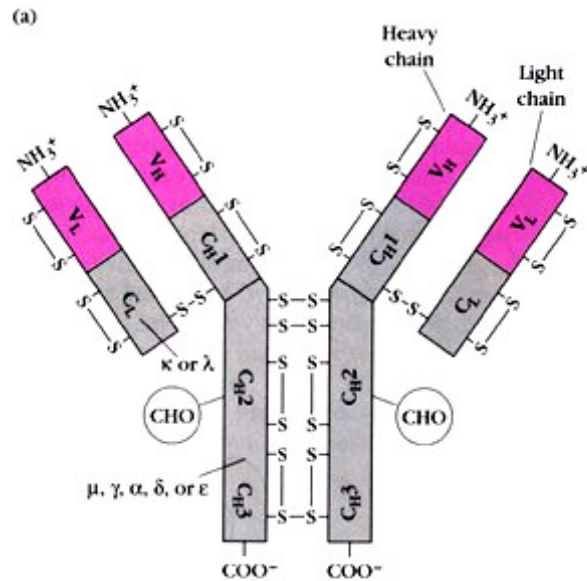
Gyógyszerészi biotechnológia

Immunglobulin szerkezet

**Ellenanyagok előállítása, tisztítása és
jelölése**

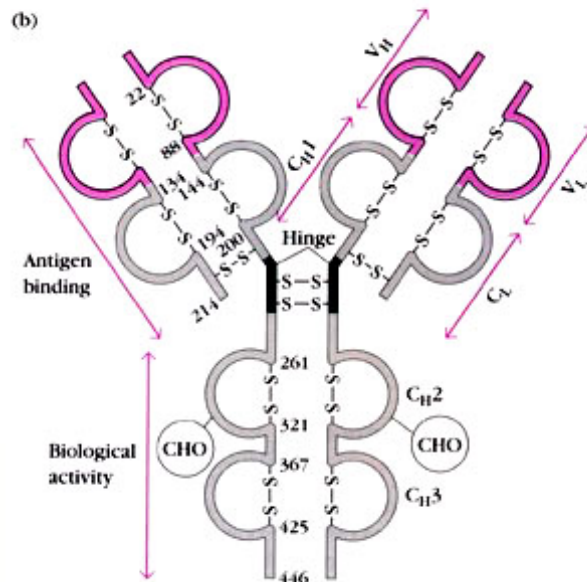
Rekombináns ellenanyagok

Az immunglobulinok szerkezete I



- 2L lánc
- 2H lánc
- Diszulfid-hidak
- Variábilis, Konstans régiók
- Antigén kötő régió: Fab

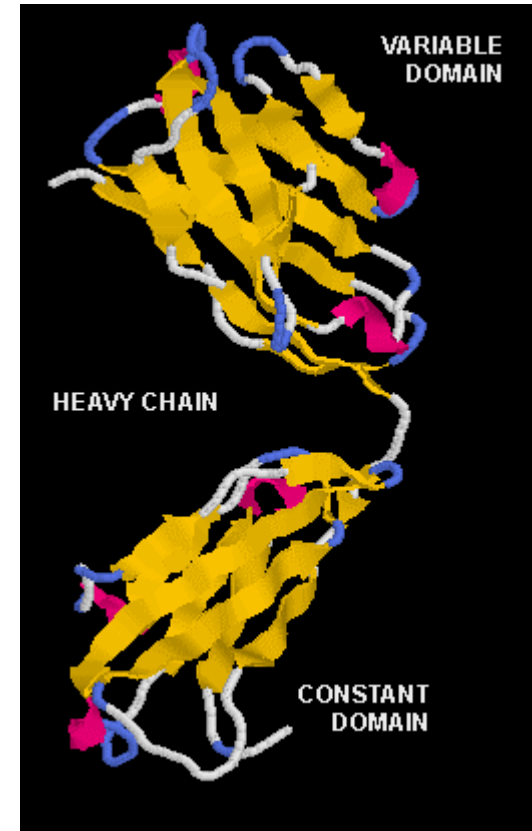
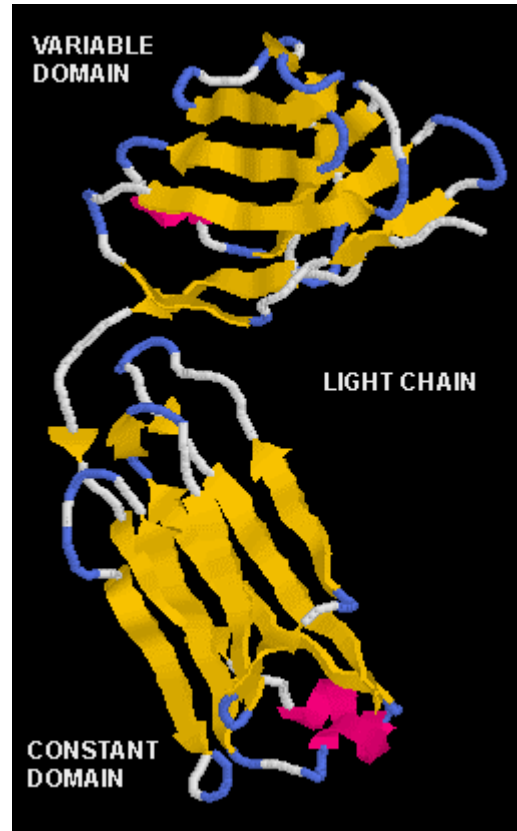
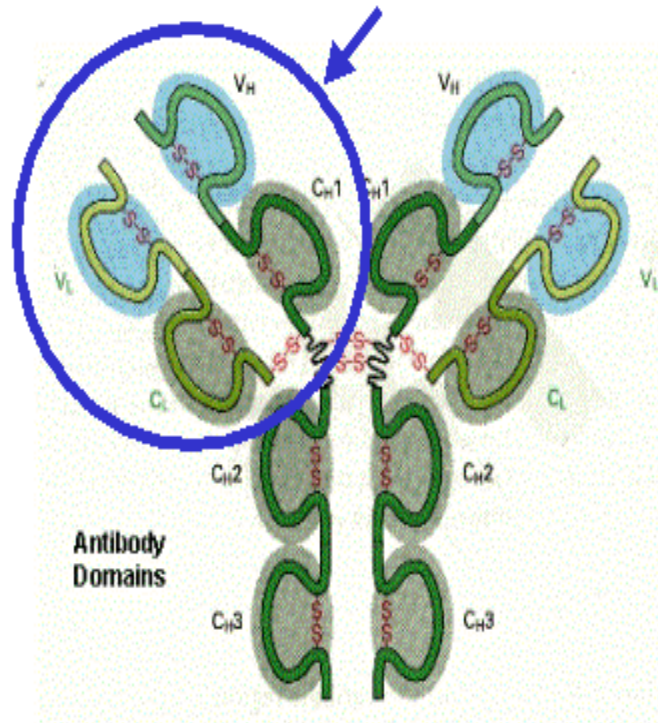
Fc



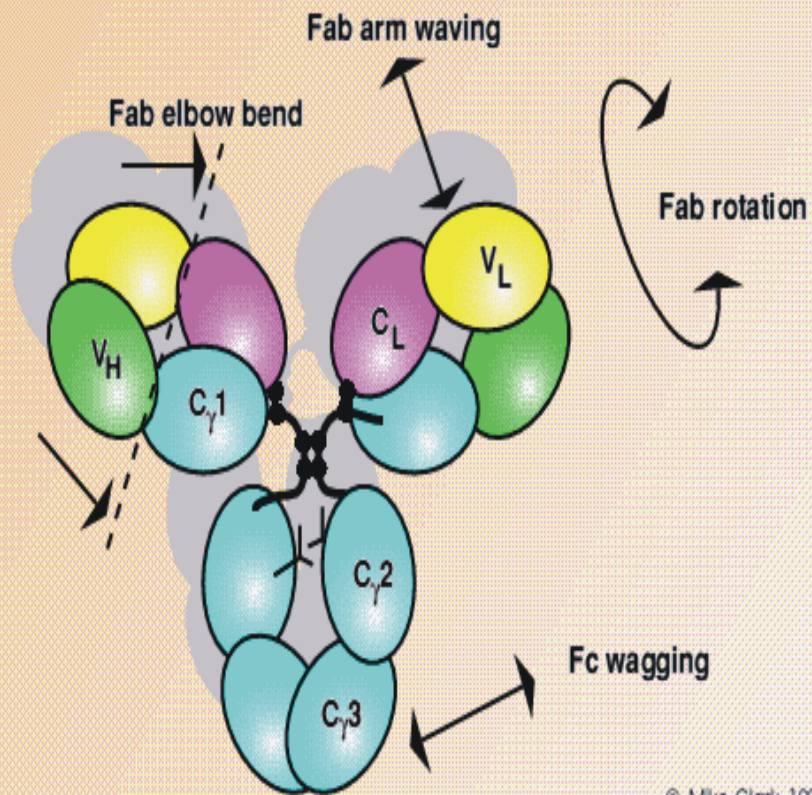
- CDR régió, komplementaritást meghatározó régió

Az immunglobulinok szerkezete II

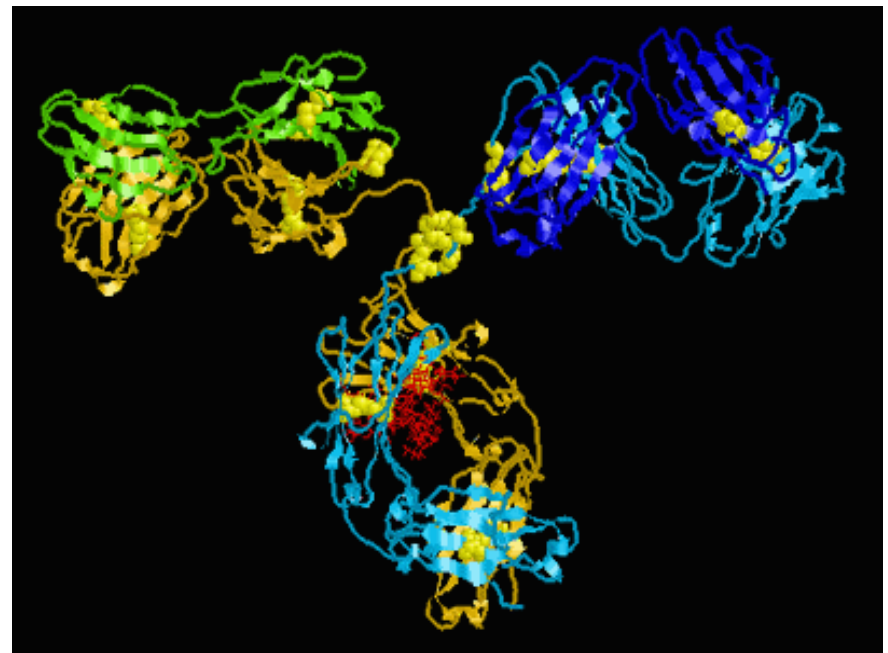
This is the Fab fragment



The IgG Molecule

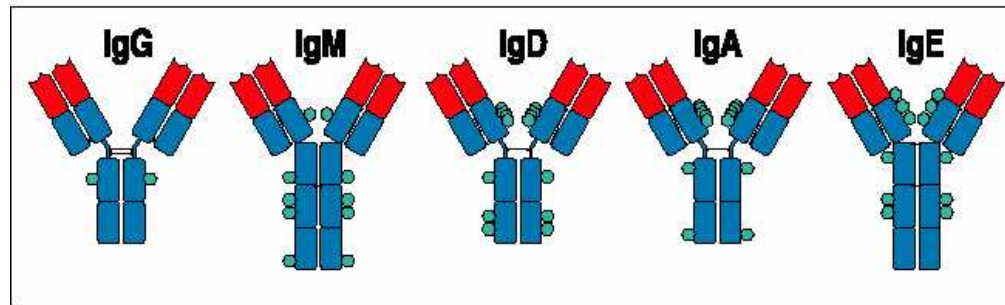


© Mike Clark 1994

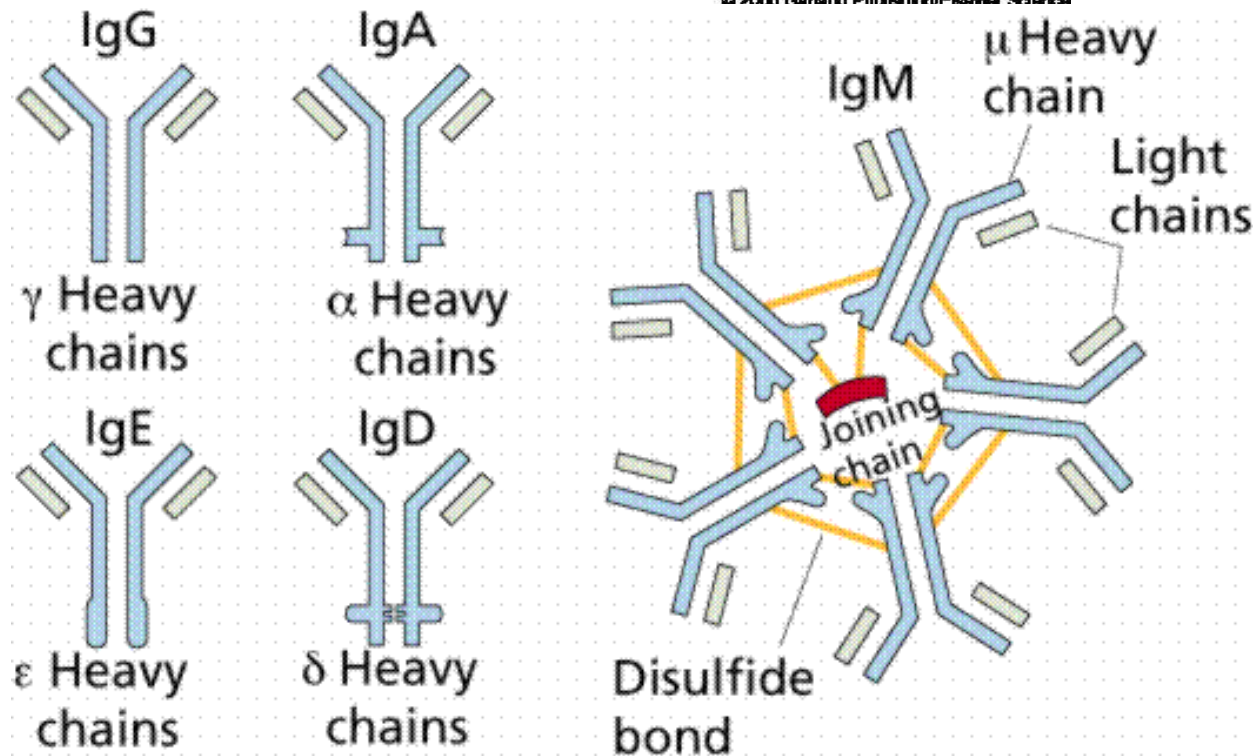


Ig típusok

Figure 24



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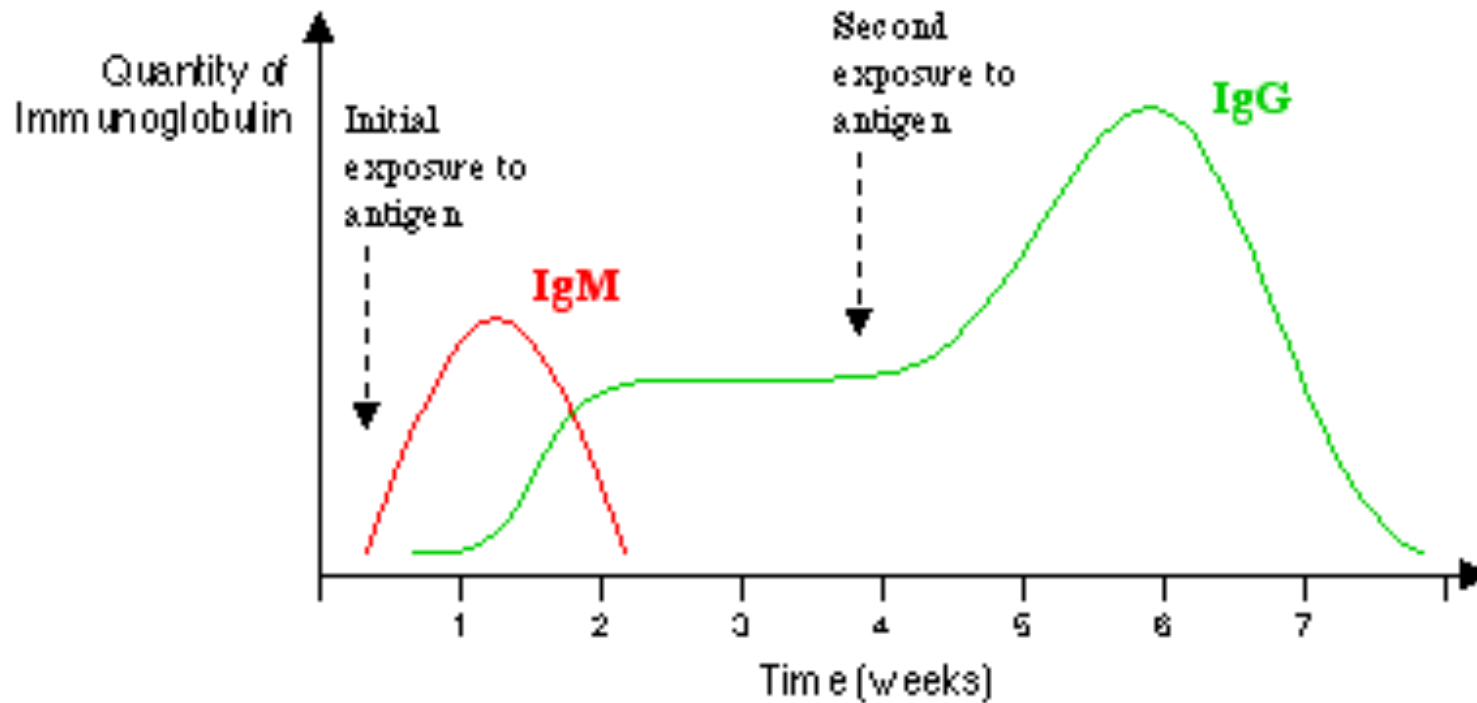
Antitestestek előállítása

- Poliklonális ellenanyagok - antiszérumok
immunizálás
ellenanyag tisztítás
jelölés
- Hybridómák és monoklonális ellenanyagok
ellenanyag tervezés és előállítás
fermentáció
jelölés

Immunizálás



Az immunglobulin termelődés kinetikája



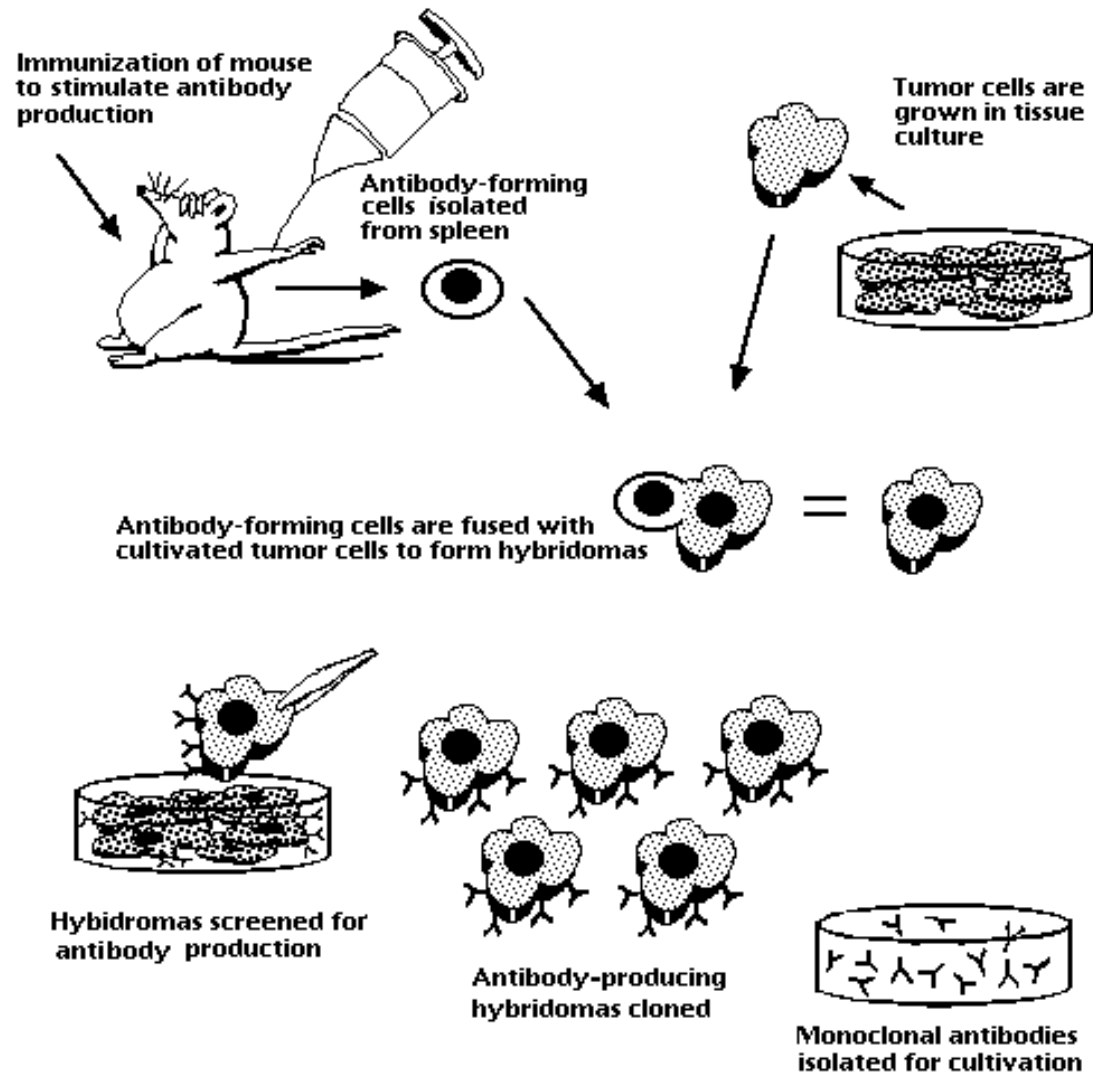
Az ellenanyagtermelést befolyásoló tényezők

- a recipiens
- az antigén természete
- az antigén dózisa
- az antigén bejuttatásának helye
- adjuvánsok
- az ismételt immunizálás kinetikája

A poliklonális ellenanyagok jellemzői

- vérszérum (változatos idiotípusú, izotípusú és eltérő affinitású ellenanyagok keveréke)
- az aviditással jellemezhetők
- standard, ameddig az adott készlet tart

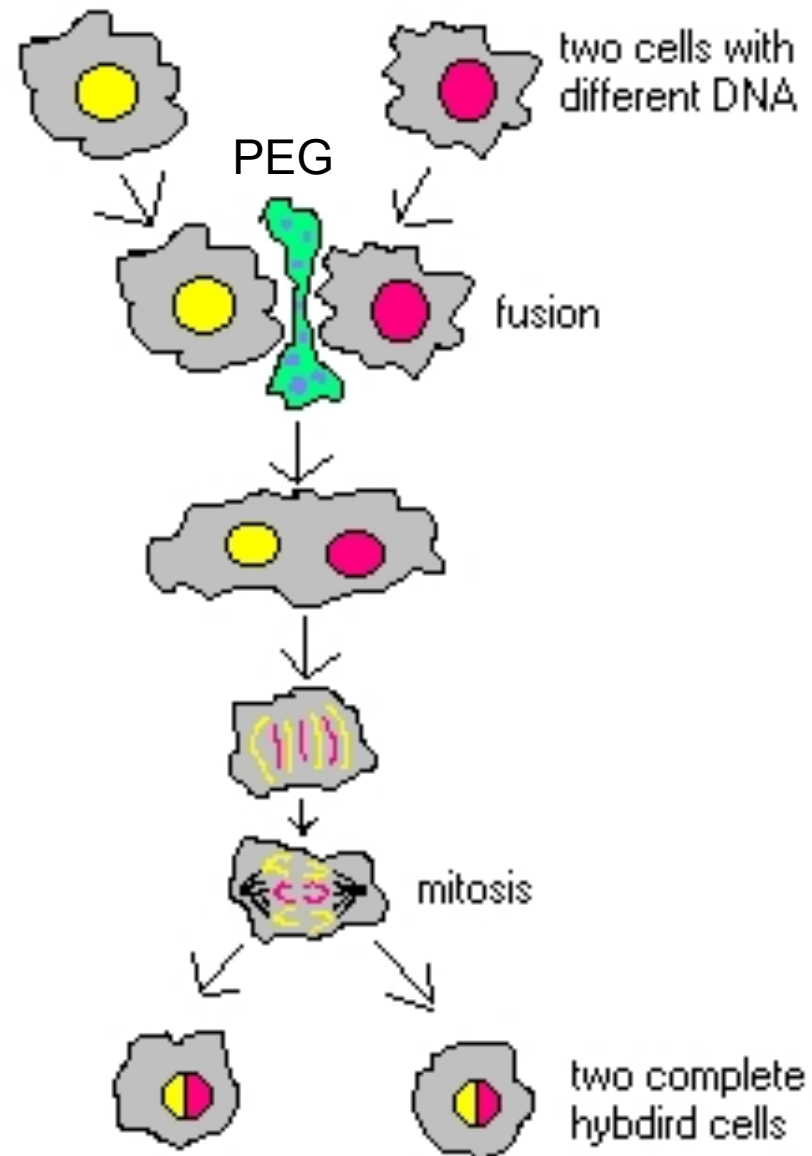
Monoclonal Antibody Production

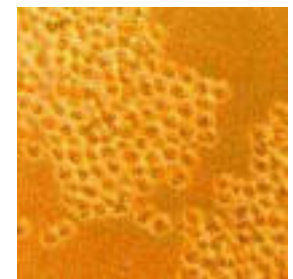
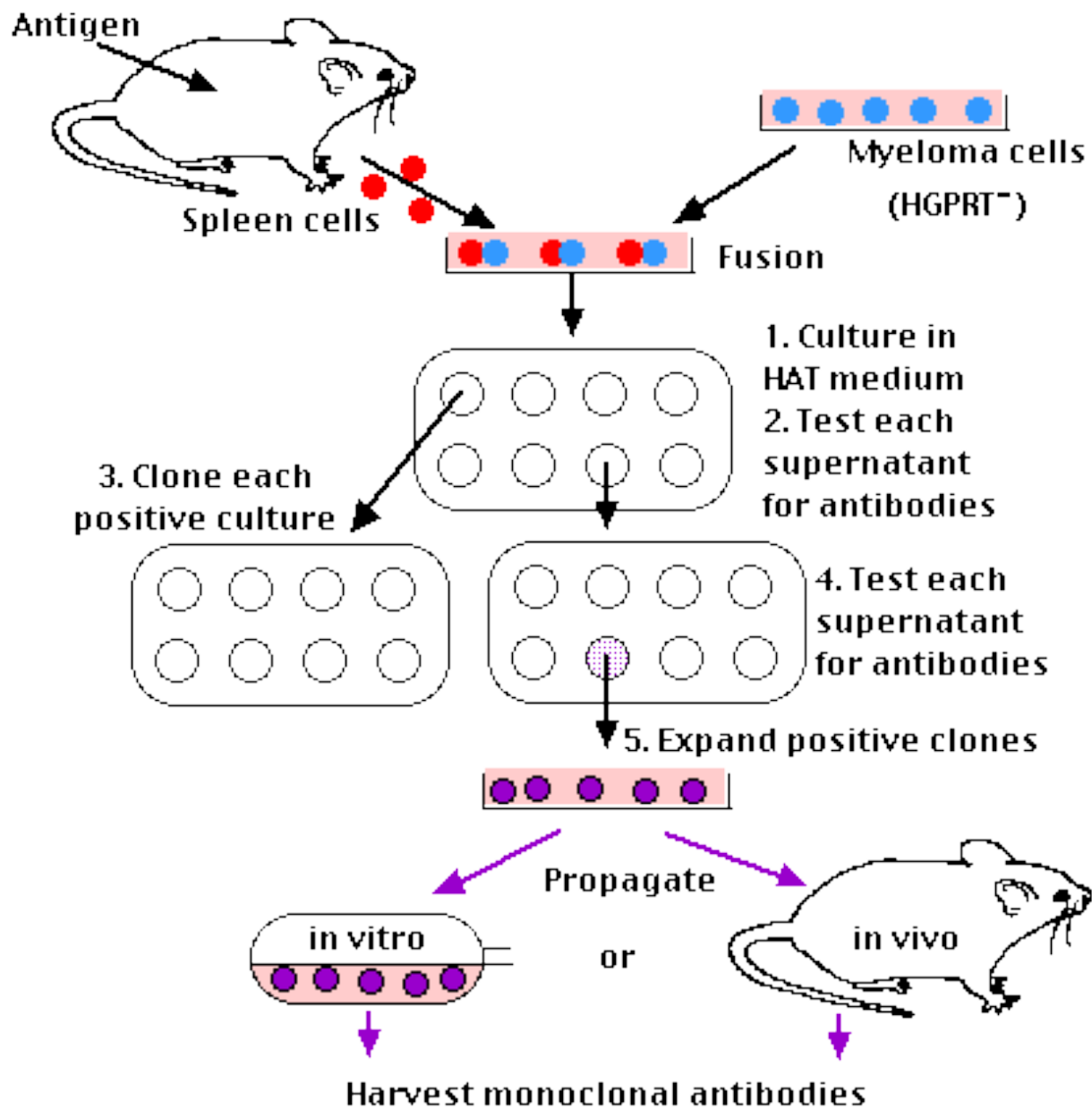


A monoklonális ellenanyagok jellemzői

- génszintézeti úton előállított ellenanyagok
- egyetlen epitópra specifikus, teljesen azonos ellenanyag molekulák
- valódi affinitással jellemezhetők
- standard, ameddig a sejtvonal fenntartható

Szomatikus sejt fúzió





Hybrid szelekció

mouse myeloma



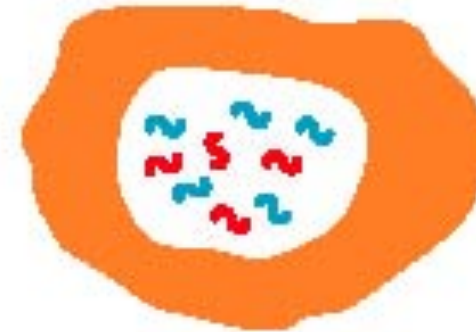
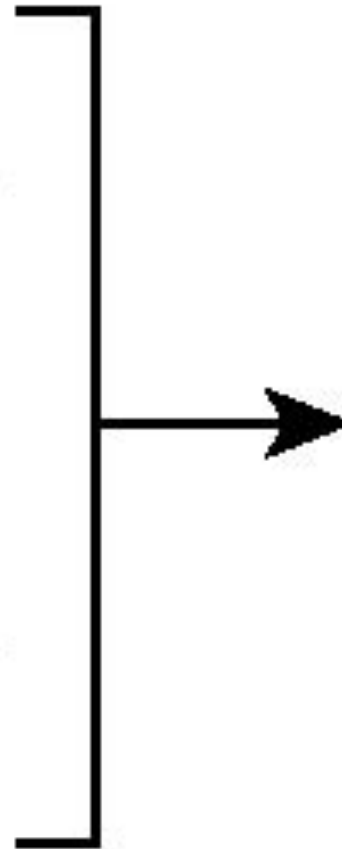
HGPRT⁻

+ PolyEthyleneGlycol



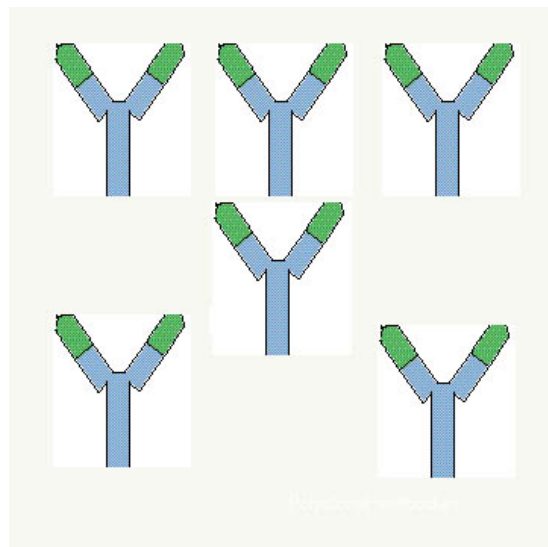
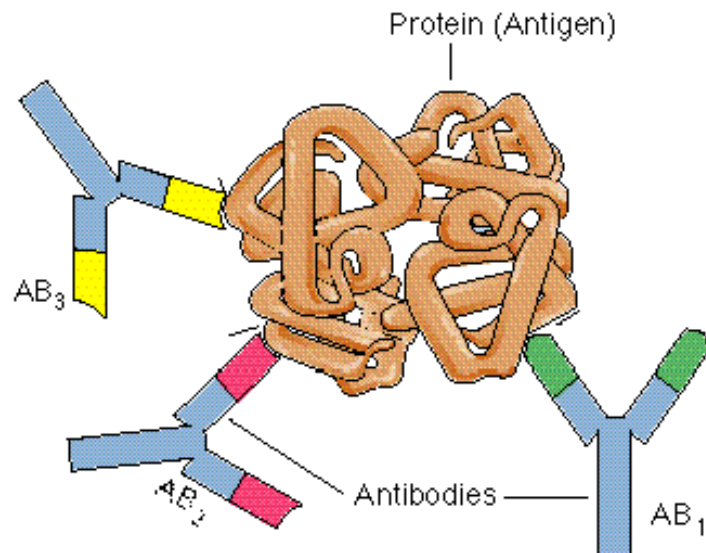
HGPRT⁺

mouse B cell
(both die in HAT)

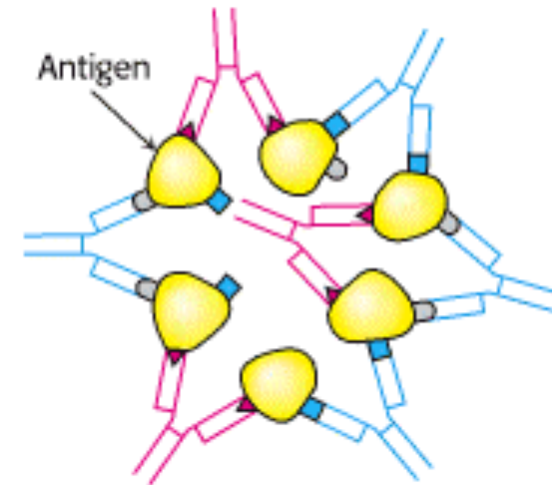


hybrid myeloma
(survives in HAT,
produces myeloma Ab
and B cell Ab)

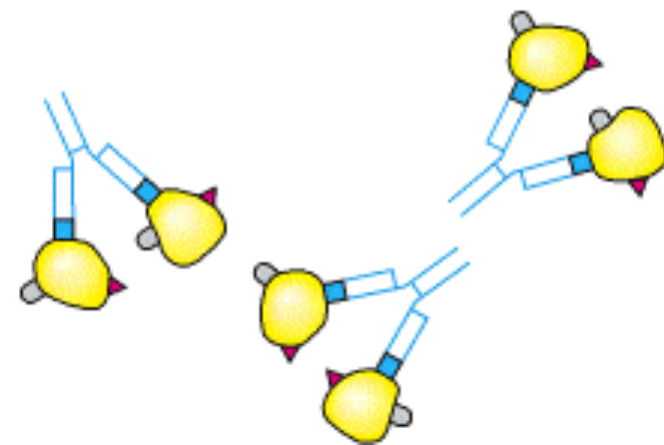
Poliklonális / monoklonális



Polyclonal Antibodies



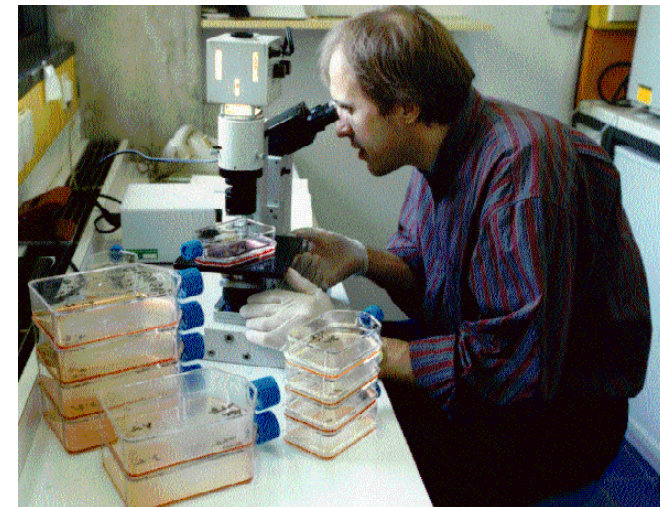
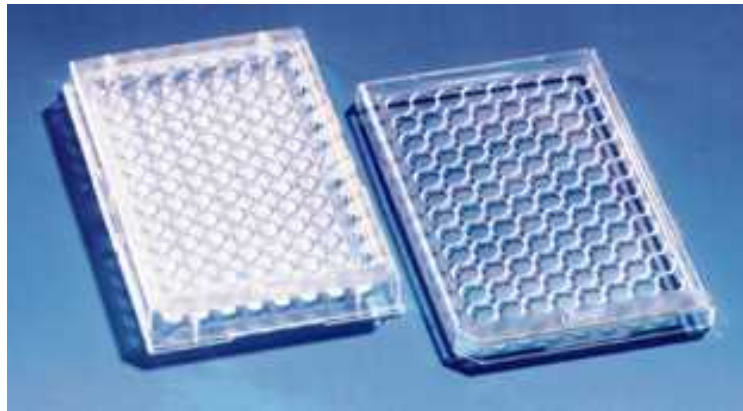
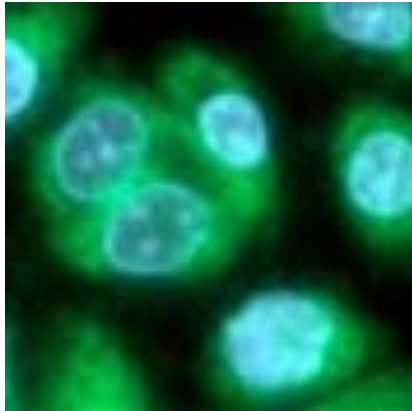
Monoclonal Antibodies



Sejt fermentálás



Hybridóma tenyésztés



Immunglobulin tisztítás

Kisózás $(\text{NH}_4)_2\text{SO}_4$ kicsapás

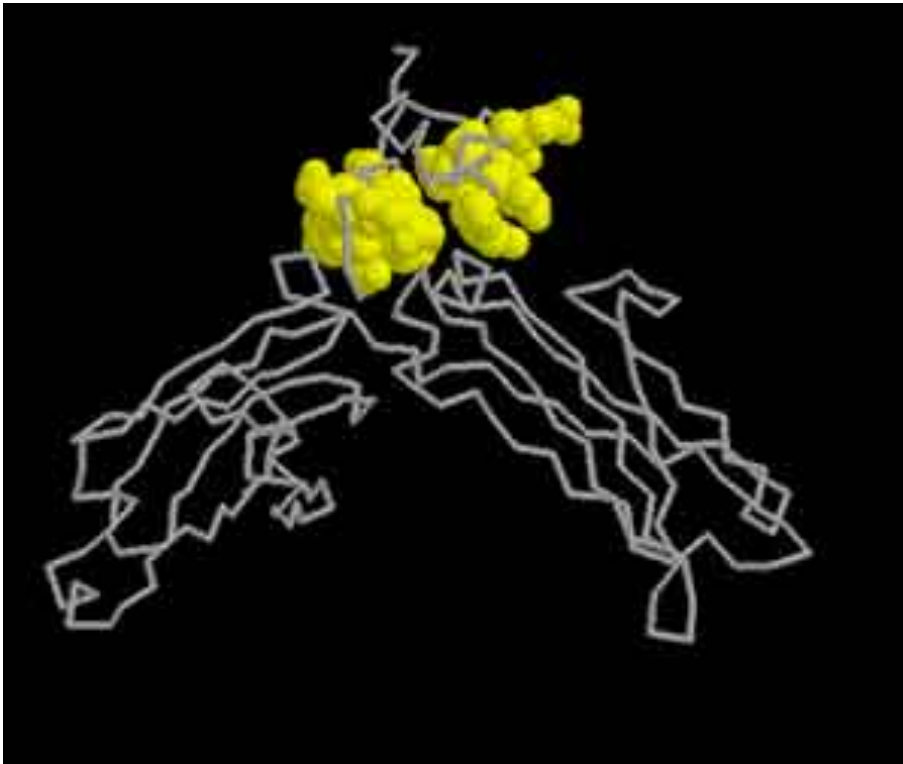
Folyadékkromatográfia

Affinitás kromatográfia (Fc vég, antigén)



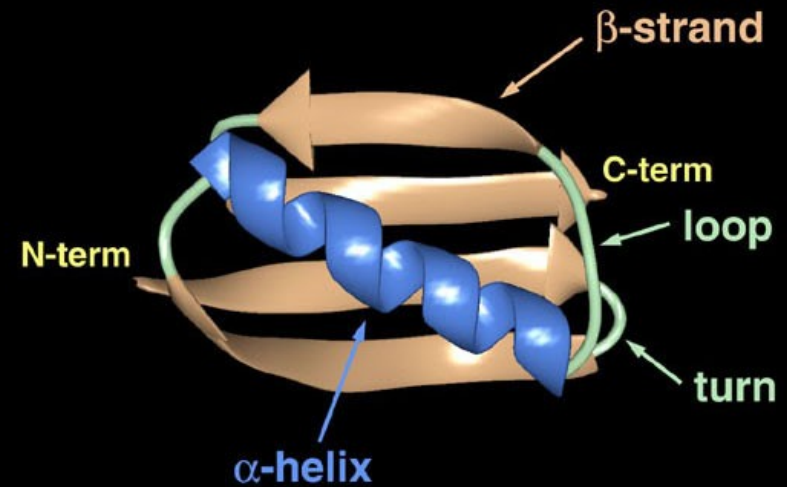
Affinitás tisztítás

Protein A

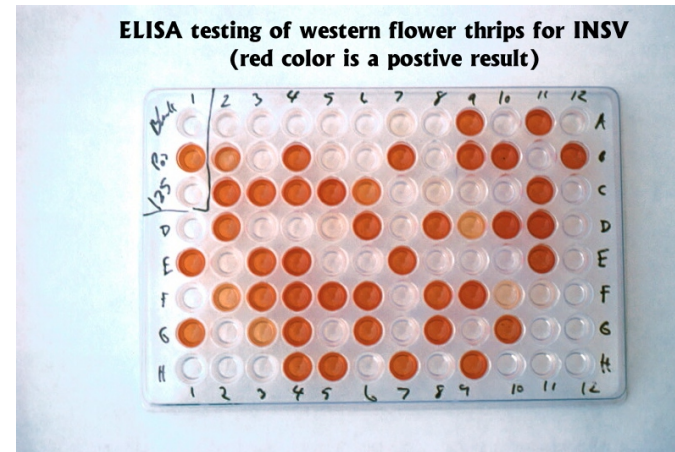
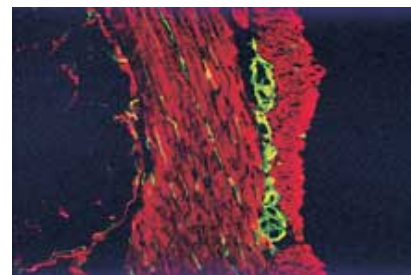
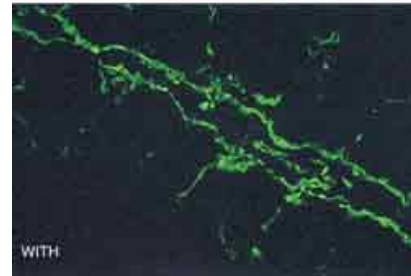
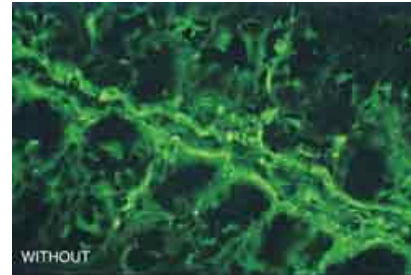
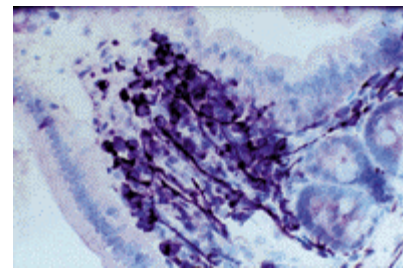
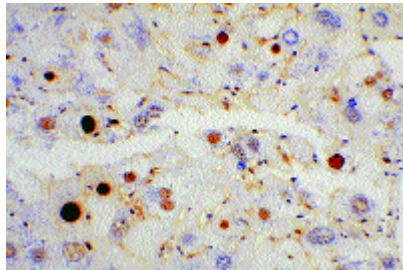
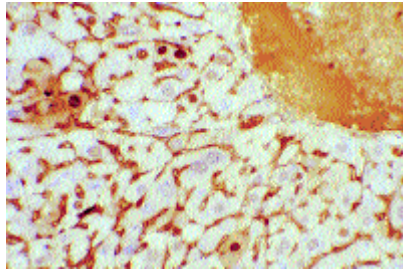


Protein G

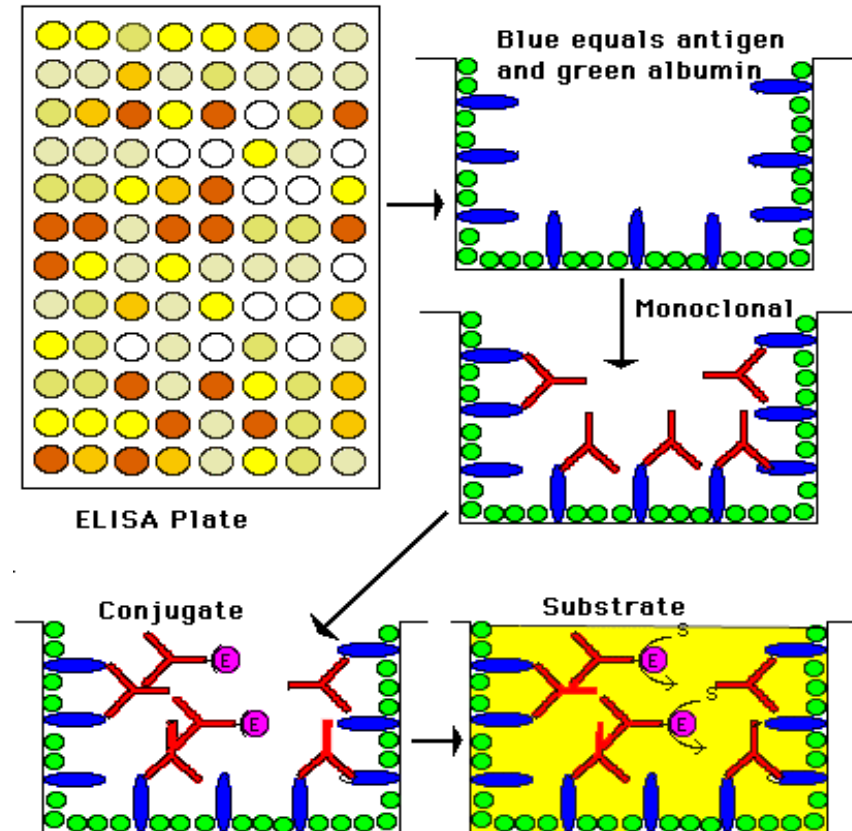
immunoglobulin binding domain of protein G



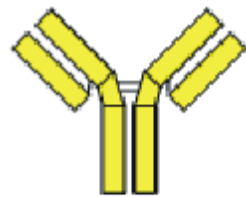
Gyakorlati felhasználás



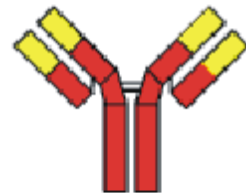
Enzyme Linked Immunosorbent Assay (ELISA)



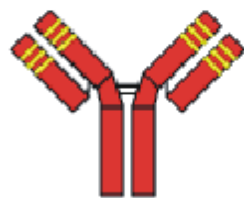
Humanizált ellenanyagok



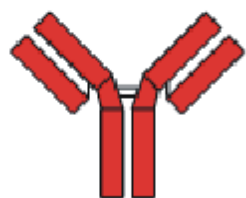
Murine



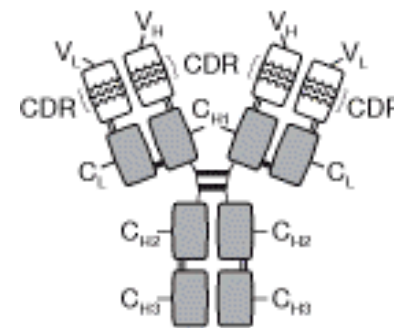
Chimaeric



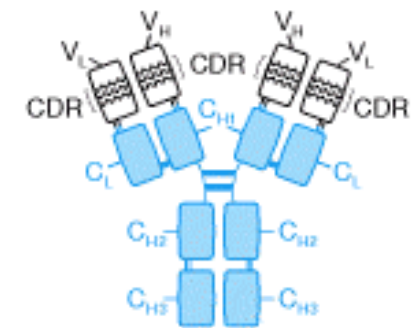
Humanised



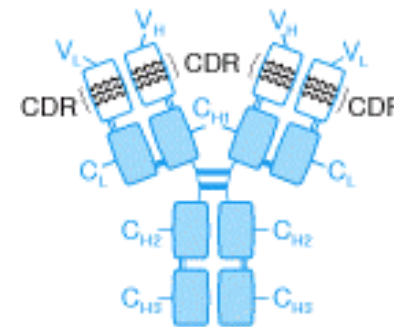
Human



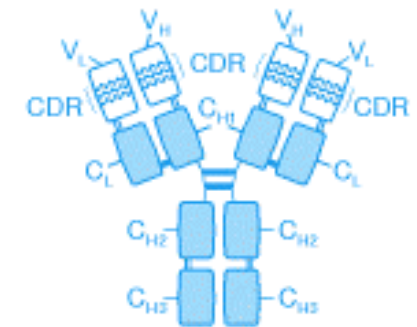
All-rodent antibody



Chimeric (V/C) antibody

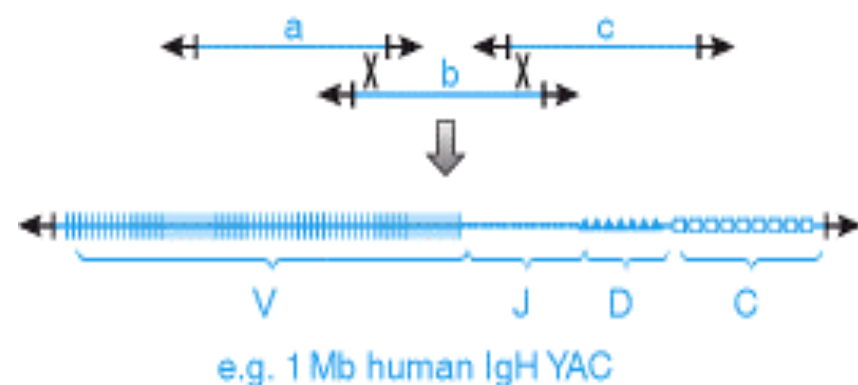


Humanized antibody

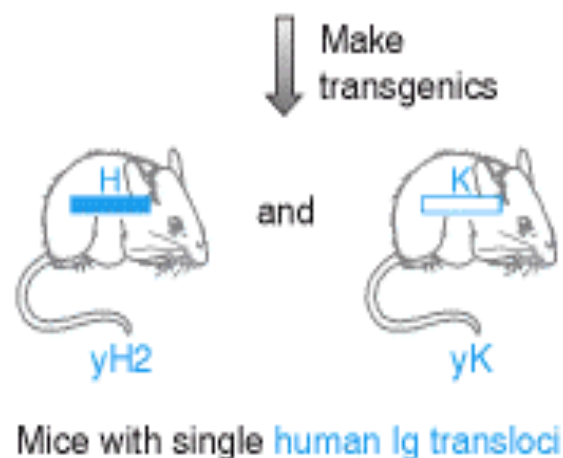


Fully human antibody

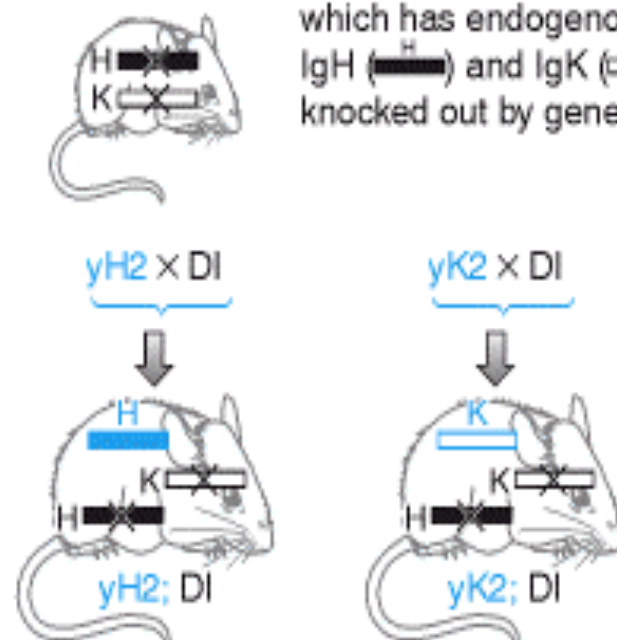
1. Isolate YACs containing sequences from human IgH (H) and human IgK chain (K) loci
2. Allow YACs from each locus to undergo homologous recombination in yeast cells to generate YACs with large inserts



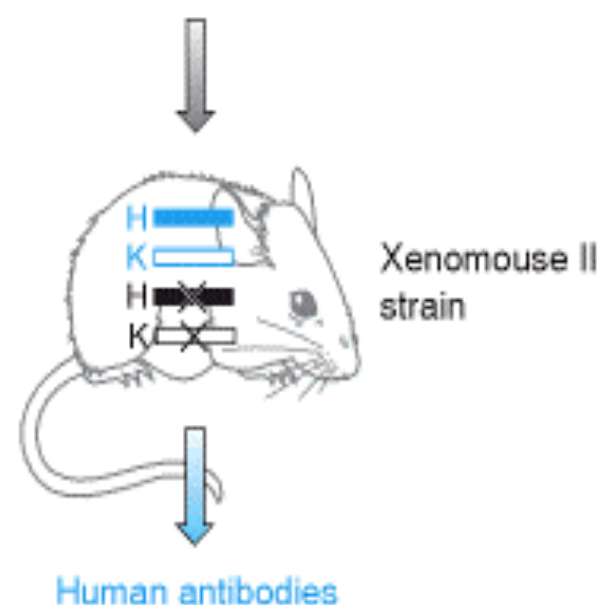
3. Fuse yeast cell spheroplasts containing large IgYAC to mouse ES cells

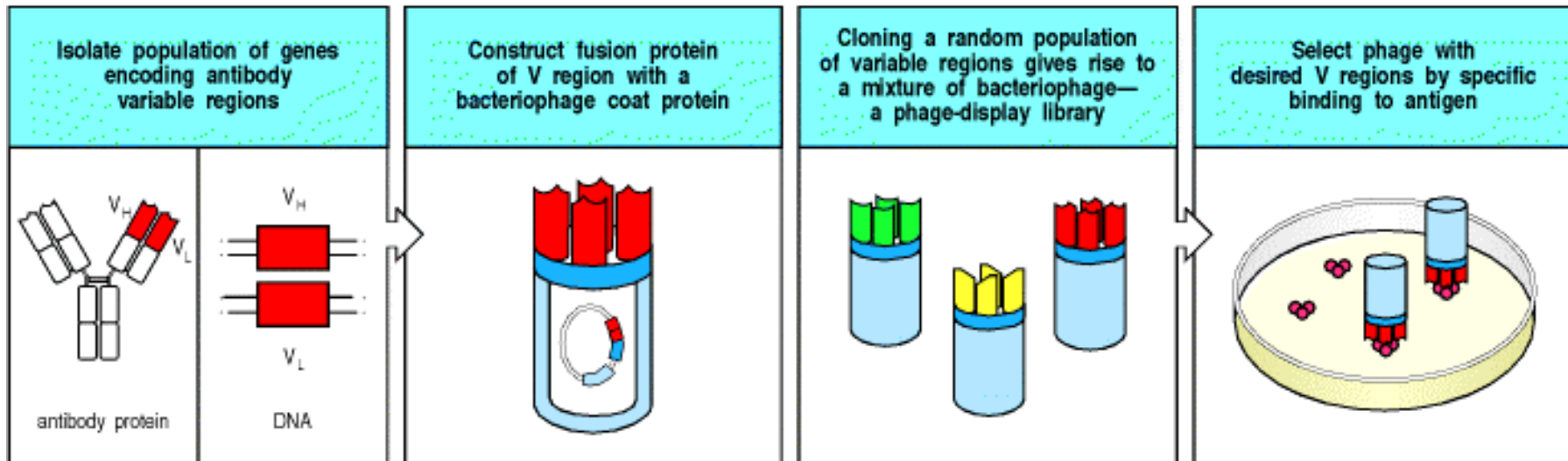
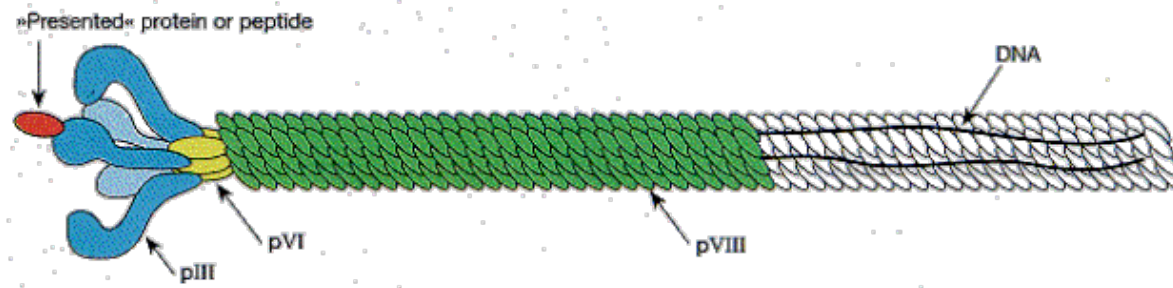


4. Cross these mice with the DI strain which has endogenous IgH (H) and IgK (K) loci knocked out by gene targeting

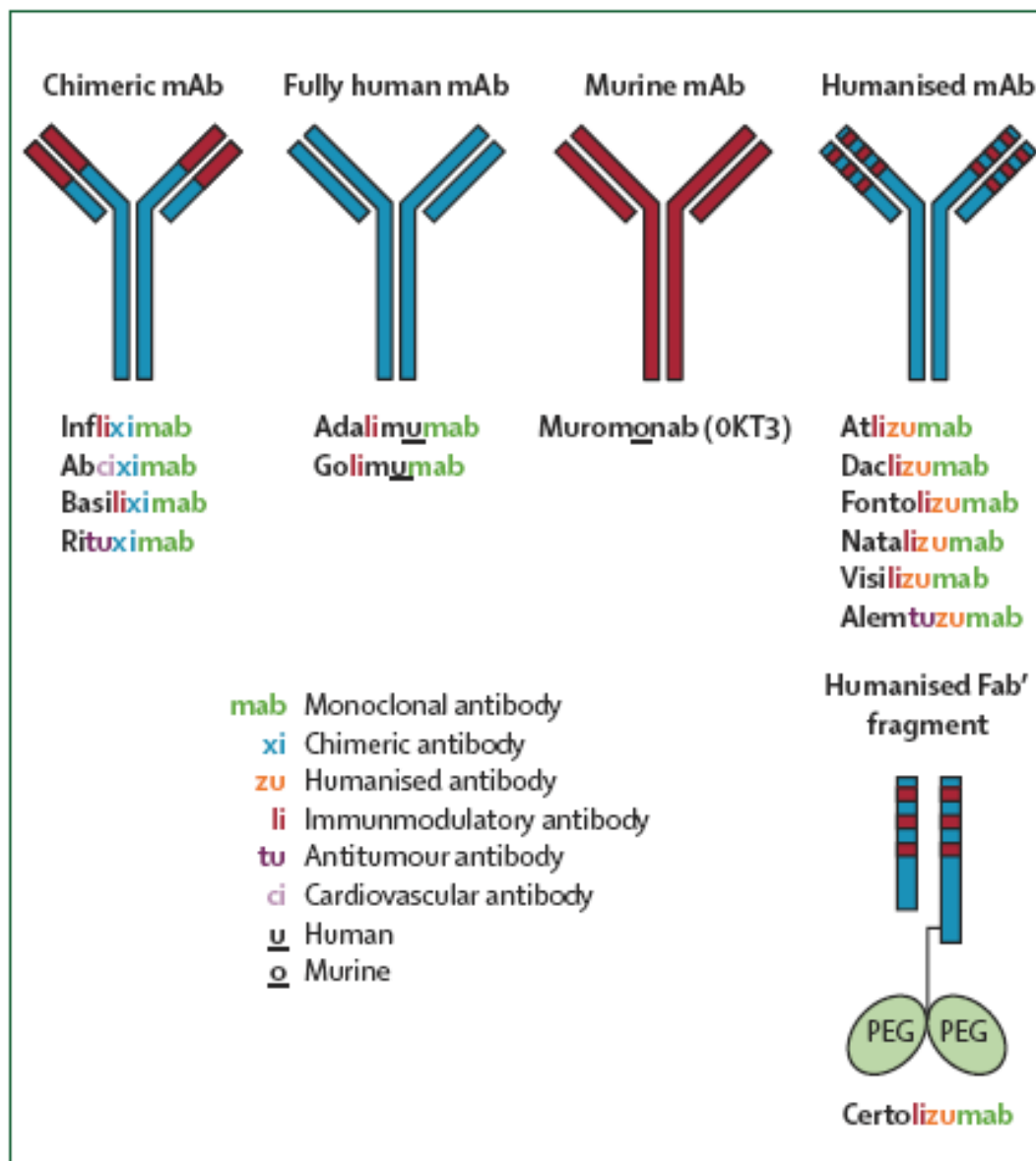


5. Cross $yH2; DI$ to $yK2; DI$





Therapiás célokra használt monoklonális ellenanyagok



Terápiás célokra használt monoklonális ellenanyagok

SCA Pipeline

Institution/Indication	Pre-Clinical	Phase I	Phase II	Phase III	Marketed
Alexion Pharmaceuticals*					
Cardiopulmonary	██████████	██████████	██████████		
Myocardial Infarction	██████████				
Royal Free Hospital/Cancer	██████████				
Sloan-Kettering/Cancer	██████████				
NIH/Cancer	██████████				
NIH/Cancer	██████████				
NIH/Cancer	██████████				
Cell Genesys*/Colon Cancer	██████████				
Novopharm Biotech/ Progressive NHL	██████████				
University of Alabama/ Cancer	██████████				
Seattle Genetics*/ Cancer (BR96 SCI)	██████████				
Dana-Farber/AIDS	██████████				

* Indicates SCA Licensee

MAB	Antibody Type	Approved Indication(s)	Year Approved
1. Orthodone OKT3 (Muromonab -- CD3; Johnson & Johnson; OrthoBiotech)	Murine	Treatment of acute kidney, heart, and liver transplant rejection	1986
2. ReoPro (Abciximab -- Centocor)	Chimeric (Hu-mu)	Adjunct to coronary intervention (angioplasty, stent, atherectomy) for prevention of coronary thrombosis	1994
3. Rituxan (Rituximab -- IDEC; Genentech)	Chimeric (Hu-mu)	Non-Hodgkin's B cell lymphoma (low-grade or follicular)	1997
4. Zenapax (Daclizumab -- Protein Design Labs; Hoffman-La Roche)	Humanized (Hu-mu)	Prevention of acute kidney transplant rejection	1997
5. Simulex (Basiliximab -- Novartis)	Chimeric (Hu-mu)	Prevention of acute kidney transplant rejection	1998
6. Synagis (Palivizumab -- MedImmune)	Humanized (Hu-mu)	Prevention of respiratory syncytial virus infection in pediatric patients	1998
7. Remicade (Infliximab -- Centocor)	Chimeric (Hu-mu)	Crohn's disease	1998
8. Herceptin (Trastuzumab - Genentech)	Humanized (Hu-mu)	Metastatic breast cancer	1998
9. Mylotarg (American Home Products)	Humanized (toxin-linked)	Acute myelogenous leukemia	2000
10. Campath (Millennium Pharmaceuticals)	Humanized	Chronic lymphocytic leukemia	2001
11. Zevalin (IDEC Pharmaceuticals)	Chimeric (Hu-Mu; radionuclide-linked)	Non-Hodgkins lymphoma	2002

Terápiás célra használt monoklonális ellenanyagok az USA-ban 1986-2001

Table 3. Therapeutic monoclonal antibodies approved by the US FDA

Generic name	Trade name	Sponsor company	Type	Approval date
Muromonab-CD3	Orthoclone	Ortho Biotech	Murine	1986
Abciximab	ReoPro	Centocor	Chimeric	1994
Rituximab	Rituxan	Genentech	Chimeric	1997
Daclizumab	Zenapax	Hoffman-La Roche	Humanized	1997
Basiliximab	Simulect	Novartis	Chimeric	1998
Palivizumab	Synagis	MedImmune	Humanized	1998
Infliximab	Remicade	Centocor	Chimeric	1998
Trastuzumab	Herceptin	Genentech	Humanized	1998
Gemtuzumab ozogamicin	Mylotarg	Wyeth-Ayerst	Humanized	2000
Alemtuzumab	Campath	Millennium/ILEX	Humanized	2001

2004: több, mint 400 áll engedélyeztetés alatt, csak az USA-ban!

