Immunology & ELISAs

2013 BIOMAN Workshop, MC³

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Show N Tell & freebies at end of workshop 😊
Disclaimers

➢ I am NOT an immunologist

➢ Nobody is paying me!

Killer T cells attacking a cancer cell
Workshop Content

• Immunology & ELISAs
  – General Overview
  – Resources
    • Commercial Kits
      Bio-Rad, Edvotek, Carolina
    • On-line sites
      Simulations, Animations
  – Hands-on Component
    • Kits: Bio-Rad & Edvotek
      Indirect ELISA
    • Multi-plate Reader
      Quantitative Assay

• Miscellaneous
  – E-Gels
  – Freebies

"it's a new strain - chicken noodle pox."
I can't say I'm entirely pleased with my hip replacement.

We've run out of lab rats, Henderson... Put this on and come with us.
NCI's Understanding The Immune System
Organs of the Immune System

- Tonsils and adenoids
- Lymph nodes
- Lymphatic vessels
- Thymus
- Lymph nodes
- Spleen
- Peyer’s patches
- Appendix
- Lymph nodes
- Lymphatic vessels
- Bone marrow

Understanding the Immune System
NIH, NIAID, NCI
booklet/tutorial:

Ab-mediated Acquired Immunity 2° Response
A Picture is worth a 1000 words!
Home-made Simulations

• Connecticut’s BioBus
  – BPB pH indicator ★
  – Coat with Tween or dish detergent

Univ of Calif Davis
Biotechnology in the Classroom:
“Disease Epidemic—ELISA Technique”
  • TMB substrate/Streptavidin peroxidase

Internet Animations

• www.biology.arizona.edu/immunology/activities/elisa/main.html
• www.sumanasinc.com/webcontent/animations/content/ELISA.html
• www.edumedia-sciences.com/en/a543-direct-enzyme-linked-immunosorgent-assay-elisa
  ➢ $8 for download
Immunology/ELISA/HIV


http://www.pbs.org/wgbh/nova/aids/textindex.html


http://www.learner.org/channel/courses/biology/units/hiv/images.html
Commercial Kits

Bio-Rad’s ELISA Immuno Explorer Kit

Edvotek’s AIDS Kit

Carolina’s ELISA Simulation Kit

$104.95 (Reagent refill $46.95)
Storage: --20°C
(or 4°C for 1 month)
Purple/Green {no Ab/Ag}
No pre-lab reagent prep
16 pairs of students
No washing steps

Used 16 months past expiration; great results (even with ½ volumes so x2 the # rxns)
Commercial Kits

Bio-Rad’s ELISA Immuno Explorer Kit

- $162.50 (Reagent refill $120)
- Storage: 4°C
- HRP/TMB blue
- Pre-lab reagent prep
- 12 student groups
- Invert strips
- Color-coding of reagent tubes
- Protocols I, II, III
- Quant option {655nm}

Edvotek’s AIDS Kit

- $99
- Storage: 4°C
- Peroxidase/brown
- Pre-lab reagent prep
- 10 student groups
- Transfer pipet removal
<table>
<thead>
<tr>
<th>Protocol</th>
<th>Type of ELISA</th>
<th>Real-World Application</th>
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<tbody>
<tr>
<td>I</td>
<td>Tracking outbreaks of disease by detecting antigens</td>
<td>HIV, SARS, Smallpox, anthrax</td>
</tr>
<tr>
<td>II</td>
<td>Detecting antigens</td>
<td>Pregnancy, drugs, GMO, BSE</td>
</tr>
<tr>
<td>III</td>
<td>Detecting antibodies in serum</td>
<td>Lyme disease, HIV, smallpox, West Nile virus</td>
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</table>
Example: Pregnancy Test

Positive

Negative
Quantitative ELISA

Enzyme-Linked Immunosorbent Assay (ELISA)-Multi-Lingual

www.youtube.com/watch?v=RRbuz3VQ100
A 96-well ELISA plate (8 cm x 12 cm)
• The cutoff value indicating a positive result is 0.500
• Optical densities of 0.300 → 0.499 are indeterminate and need to be retested
Indirect ELISA:

To detect HIV antibodies in serum

- Inactivated HIV antigens pre-coated onto an ELISA plate
- Patient serum
- Anti-human immunoglobulin coupled to an enzyme. This is the second antibody, and it binds to human antibodies
- Chromogen or substrate which changes color when cleaved by the enzyme

ELISA

Indirect ELISA

1. Antigen/sample is added to plate.
2. Blocking buffer is added to block remaining protein-binding sites.
3. Next a suitable primary antibody is added.
4. A suitable secondary antibody – HRPO conjugate is then added which recognizes and binds to the primary antibody.
5. TMB substrate (Leinco Prod. No. T118) is added and is converted by HRPO to detectable form.

Diagram 1: Illustration of Indirect ELISA method.
Indirect ELISA

1. protein coated container
2. Antibodies bind to protein
3. Excess washed away

5. visual change confirms correct protein is present
4. second antibody binds to first

CD available from HHMI Virtual Lab:
Immunology/ELISA
Order online
"I hate it when we're not sure we're inoculating against the right strain of flu virus."

"Do I have to declare a major? Couldn't I just be a stem cell?"

"I think there's a spy among us..."

"According to all our tests, your immune system is out to lunch?"