

# **Uses of 2D gel electrophoresis in recombinant protein production**

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[www.kendricklabs.com](http://www.kendricklabs.com)

# Recombinant proteins are biologics

## ❖ **What is a Biologic?**

Biological products are those derived from natural sources. They can be composed of sugars, proteins, nucleic acids, or complex combinations, or may be living cells and tissues. Examples: flu vaccine, artificial skin, gene therapy, and especially **recombinant therapeutic proteins**. The latter are derived from cultured cells or bacteria.

## ❖ **How do biologics differ from conventional drugs?**

**Conventional** drugs have known structures and may be chemically synthesized. In contrast, biologics are complex and not easily characterized. They tend to be heat sensitive and susceptible to microbial contamination.

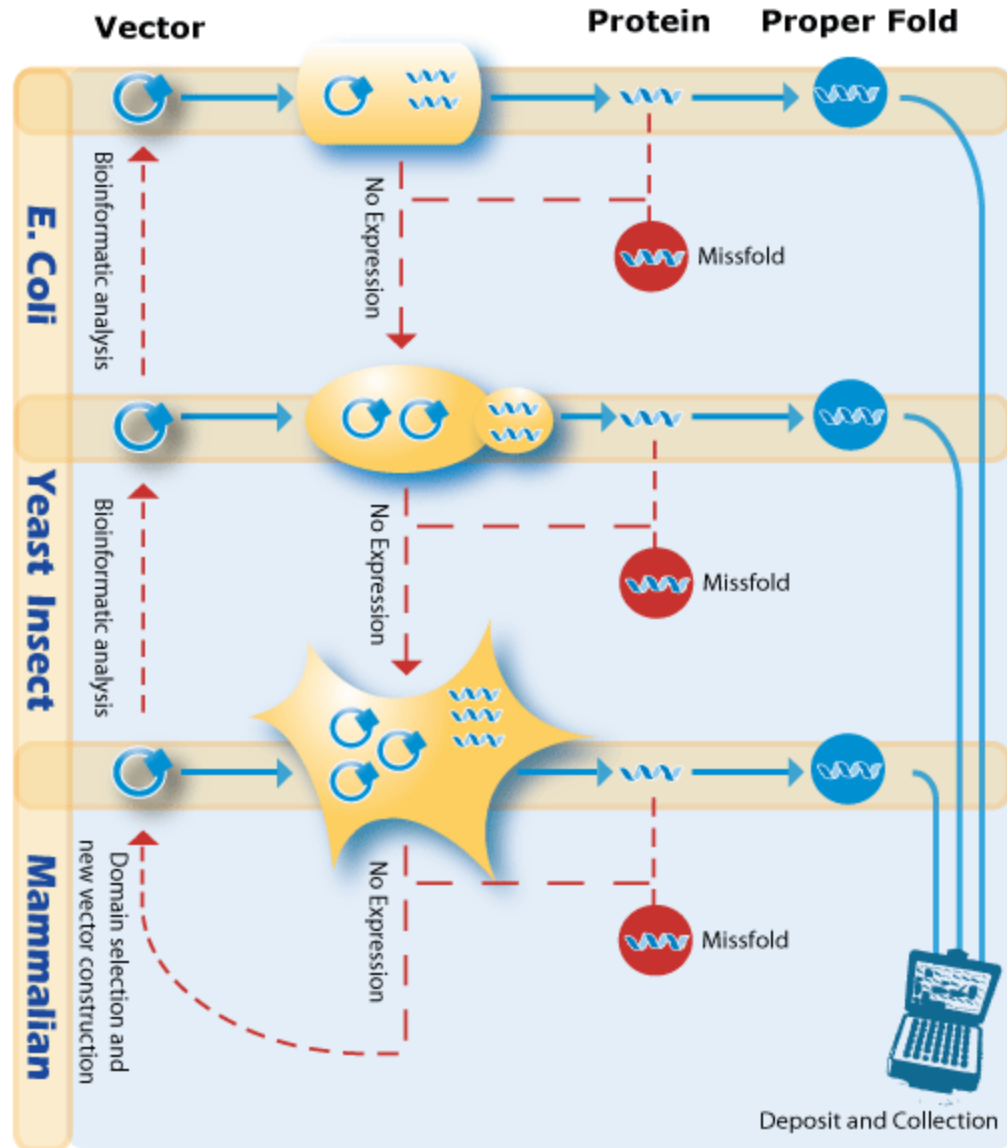
# Classes of Approved Recombinant-Protein Drugs with examples

- ❖ **Hormones:** Insulin (Humulin, the first in 1983), human growth hormone (Humatrope),
- ❖ **Cytokines:** Interferon alfa (Roferon-A), interleukin (Proleukin)
- ❖ **Clotting factors:** Factor VII (NovoSeven), factor VIII (Kogenate)
- ❖ **Monoclonal antibodies:** anti-VEGF (Avastin), anti-EGFR (Erbix)
- ❖ **Vaccine products** Hepatitis B surface antigen (Recombivax HB), *B. burgdorferi* outer surface protein A (LYMErix)
- ❖ **Enzymes:** Glucocerebrosidase (Cerezyme), DNase (Pulmozyme)
- ❖ **Novel synthetic proteins:** Fusion protein of interleukin-2 and diphtheria toxin (Ontak)
- ❖ **Novel conjugates:** Covalently attached metal chelators: (Zevalin); Covalently attached chemotherapeutics: Mylotarg

Taken from: Dudzinski, D. & Kesselheim, A., *NEJM* **358**: 832, 2008

Recombinant Protein Synthesis Diagram  
from Genway Biotech:  
[www.genwaybio.com](http://www.genwaybio.com)

Host cells can be  
*E. coli*, yeast, insect,  
and mammalian  
cultured cells,  
especially CHO

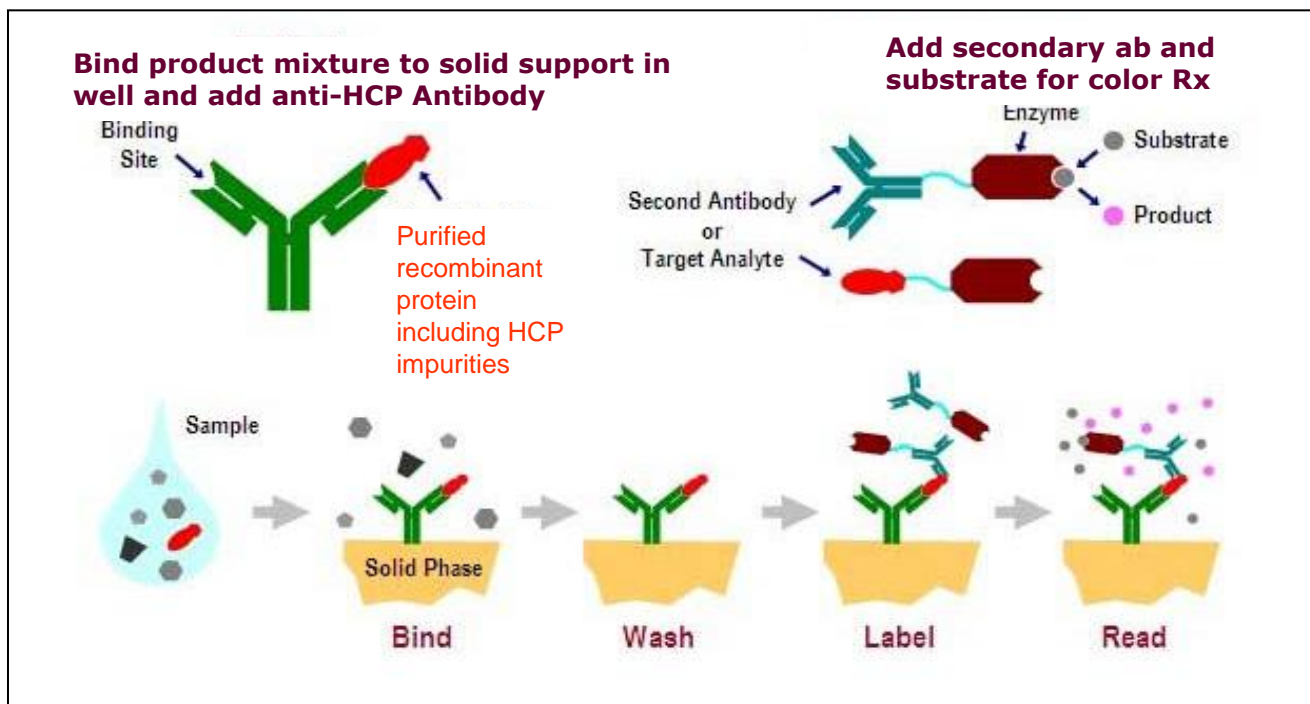


# 2D gel electrophoresis is useful at several stages of production.

- ❖ It is used to characterize charge isoforms of recombinant proteins, for example, to show glycosylation.
- ❖ 2DE in combination with western blotting (WB) or silver staining during production steps allows sensitive detection of contaminants (1)
- ❖ 2D WB may be used to characterize anti-HCP antibodies for Elisa testing.

1. Hunter, A. et. al Separation of host cell proteins oppa and dppa from recombinant apolipoprotein in an industrial hic unit operation. *Biotechnol Prog*, 2009. 25(2): p. 446-53.

To measure HCP contamination, an anti-*HCP* antibody is used to set up a quantitative Elisa assay



Elisa testing can be standardized and validated for GMP. However, it's only as good as the polyclonal antibody.

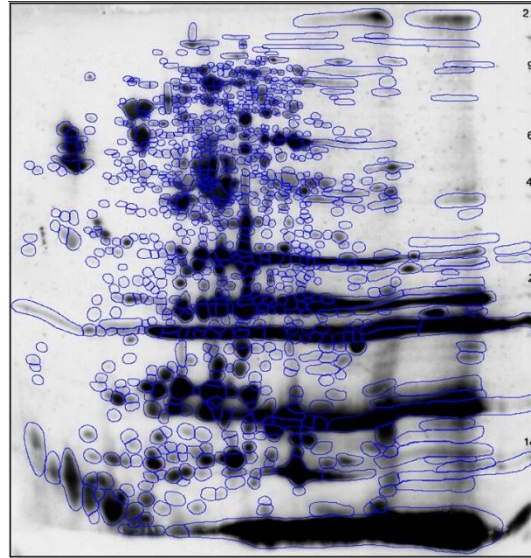
# Elisa antibodies must be checked for broad reactivity

- ❖ The Kendrick Labs team is expert at testing anti-HCP antibodies using 2D gel western blotting. The number of protein spots on a 2D WB film is compared to the number on a silver-stained pattern from the same sample. This [PowerPoint](#) provides method details.
- ❖ If the western blot shows a high percentage of the silver-stained proteins, it's a good antibody.

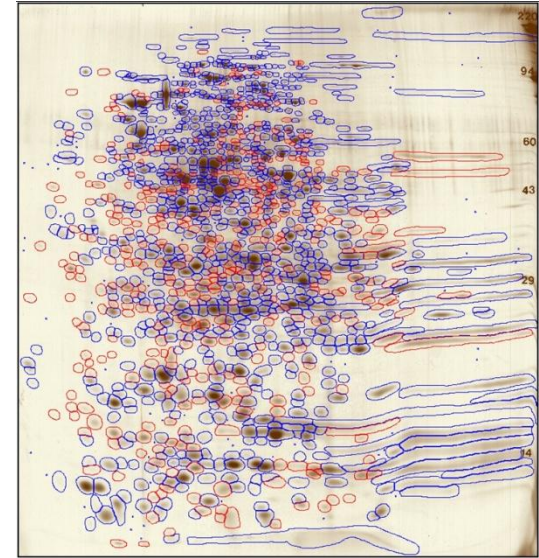
# Example: E. coli HCP antibody analysis



Silver-stained 2D gel from E. coli generic HCP sample shows 1400 protein spots.



Western blot 2D pattern from Cygnus antibody against E. coli HCP shows 993 protein spots.



Results: Blue outlines indicate spots detected in both silver and film patterns. Red shows spots only in silver. Small blue dots indicate spots detected only by Western blotting.

Final Result: 993/1400 total spots are detected by the antibody = 71% coverage. [Report Example](#)



# Key Personnel



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