Practical considerations for protein purification and sample preparation

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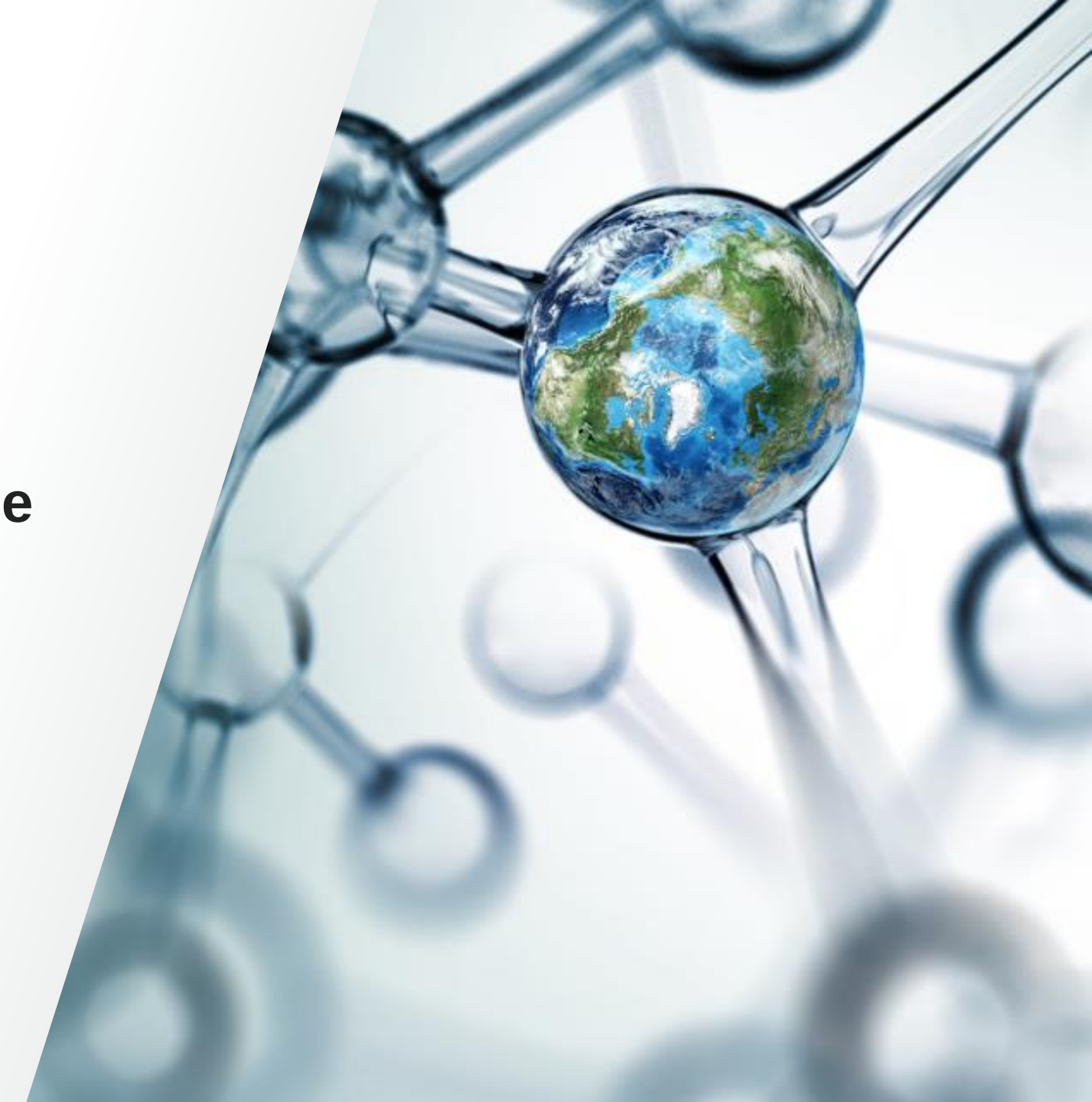
December 9, 2020



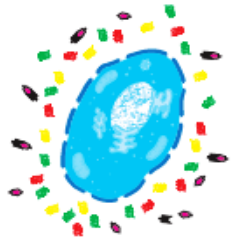
The world leader in serving science

Outline

1. Expression systems
2. Cell/tissue extraction
3. Affinity purification resins/beads
4. Automated purifications with magnetic supports
5. Protein clean-up

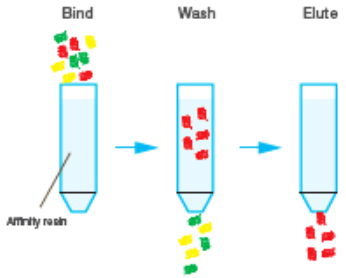
Protein sample prep workflow

1 Extraction



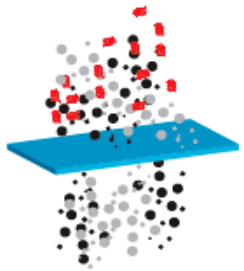
- Total protein extraction
- Subcellular fractionation
- Protease/phosphatase inhibitors

2 Purification



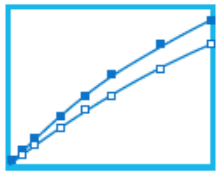
- Resins and magnetic beads
- Epitope-tag proteins
- Antibody purification
- Biotin-labeled proteins
- Strong cation/strong anion exchange

3 Clean-up



- Dialysis
- Desalting (size exclusion resins)
- Concentration

4 Quantitation



- BCA protein assays
- Coomassie dye-binding assays

5 Detection

- SDS-PAGE
- Western blot
- Mass spectrometry

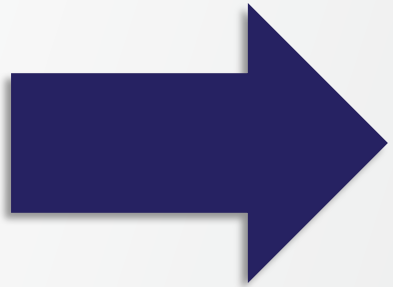
What do you want to do?

Protein isolation procedure depends upon

- **Sample type**
- **Location of protein of interest**
- **Required protein yield**
- **Proposed downstream applications**
  - Western blotting
  - Enzyme-linked immunosorbent assay (ELISA)
  - Gel shift assays (EMSA)
  - Reporter assays
  - Mass spectrometry
  - Immunoprecipitation (IP)
  - Activity assays
  - Structural biology

How should I purify it?

- How much protein do I need?
- What is my protein source?
- What model organism should I use for overexpression?
- How stable is my protein of interest?
- What sensitivities do I need to worry about?
- What downstream applications will I be doing with it?
- How pure does the protein need to be?



Purification strategy

Outline

1. Expression systems
2. Cell / tissue extraction
3. Affinity purification (resins/beads)
4. Automated purifications with magnetic supports
5. Protein clean-up

Expression system options

	Advantages	Challenges	System
Cell-free	• Rapid expression directly from plasmid • Open system, no cultures • Amenable to higher throughput	• Large-scale expression	• *E. coli* • Wheat germ • Rabbit reticulocyte • Mammalian (HeLa)
Bacteria	• Scalable • Low cost • Simple culture conditions • Short expression duration	• Protein solubility • Minimal post-translational modifications • Some mammalian proteins may not express	• *E. coli* • *Bacillus subtilis*
Yeast	• Low cost • Simple media requirements • Eukaryotic protein processing	• Fermentation required for very high yields • Growth requirements may need to be optimized	• *S. cerevisiae* • *Pichia pastoris*
Insect	• Low cost • PTMs similar to mammalian • Good for proteins toxic to mammalian cells • Expression of multi-protein complexes	• More demanding culture conditions • PTMs and folding not quite identical to mammalian systems	• Sf9 • Sf21
Mammalian	• Highest level of correct post-translational modifications • Highest probability of obtaining fully functional human proteins	• More demanding culture conditions • High yields best achieved with suspension cultures	• HEK293 • CHO

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Protein expression solutions

Gibco™ Optimized protein expression systems

Expi293™ Expression System

Structure/function studies

Why? Human cells provide native folding and post-translational modifications

Human 293 (HEK293) cell-based system
Protein yield up to 1 g/L

ExpiCHO™ Expression System

Biopharma drug discovery

Why? 70% of biologics manufactured in CHO: screen in CHO, stay in CHO

CHO cell-based system
Protein yield up to 3 g/L

Host: mammalian

ExpiSf™ Expression System

Vaccine development, academia

Why? Insect cells are a cost-effective, versatile emerging platform for recombinant vaccine production

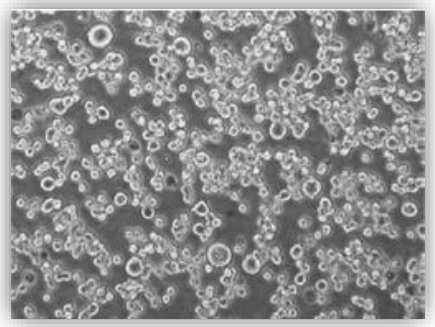
Sf9 cell-based system
Protein yield 3x greater than current platforms

Host: insect

Expi293 system

Expi293F cell line attributes

- Human cells derived from Invitrogen™ FreeStyle™ 293F cells
- Adapted for high-density culture (≥15M cells/mL)
- Doubling time of ~24-25 hours
- Cell diameter 18 - 20µm (culture – expression)
- Highest transfection efficiency (80-85%)
- Stable growth and expression profiles over 30 passages
- High quality, biologically-active protein
- Express in cells or secrete expressed protein into the media



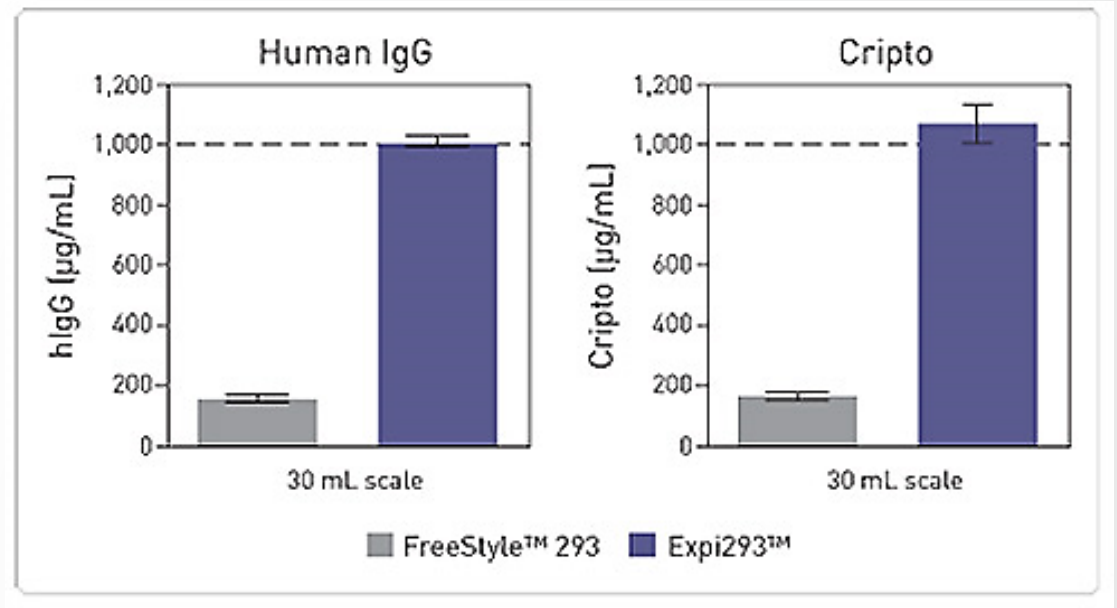


Figure 1. Expression of human IgG and Fc-tagged Cripto achieve expression levels of over 1 g/L in the Expi293 Expression System.

Expi293 expression system produces more active protein

SCIENTIFIC REPORTS
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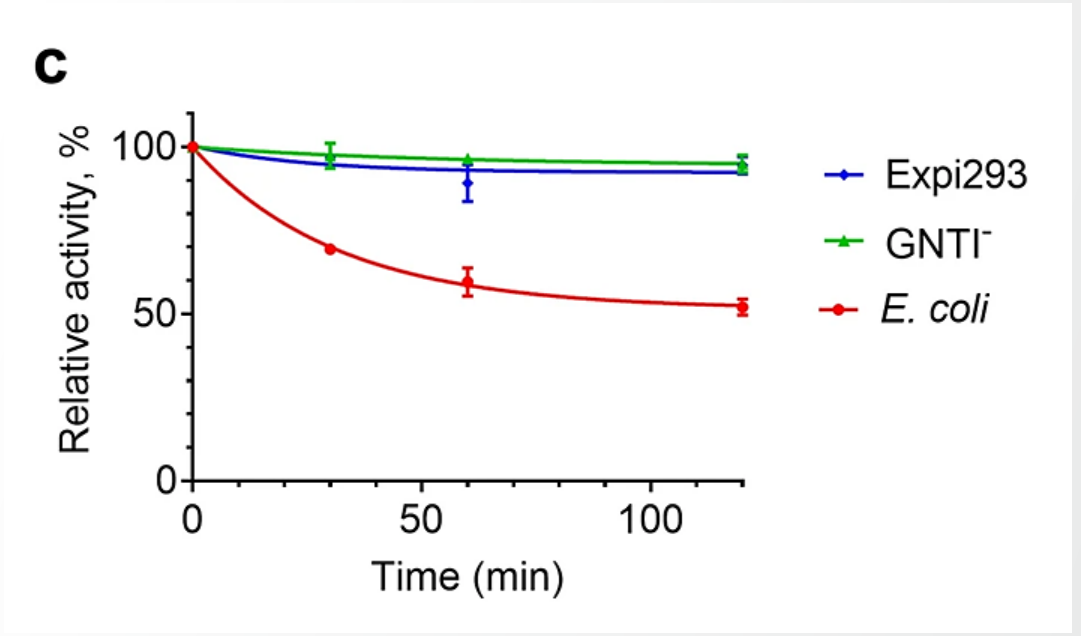
Thermostability of a recombinant G protein-coupled receptor expressed at high level in mammalian cell culture

Alexei Yeliseev[1✉], Arjen van den Berg[2], Lioudmila Zoubak[1], Kirk Hines[1], Sam Stepnowski[2], Kyle Williston[2], Wanhua Yan[2], Klaus Gawrisch[1] & Jonathan Zmuda[2]



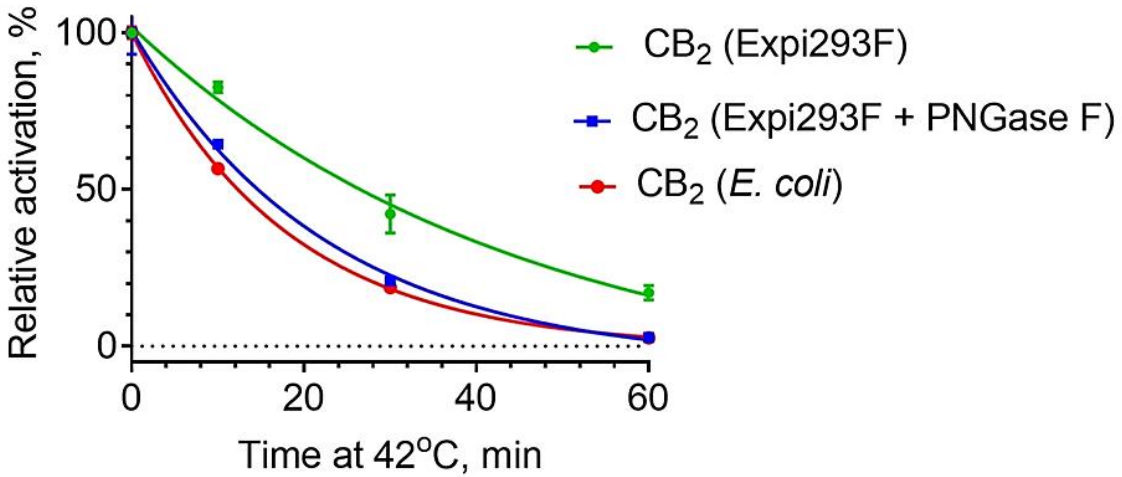
Membranes were pre-treated with 5 μM CP-55,940, then incubated at 42°C and aliquots withdrawn at time intervals indicated. Results of duplicate measurements determined by G protein activation test are presented.

Yeliseev, A., van den Berg, A., Zoubak, L. *et al. Sci Rep* **10,** 16805 (2020). https://doi.org/10.1038/s41598-020-73813-7

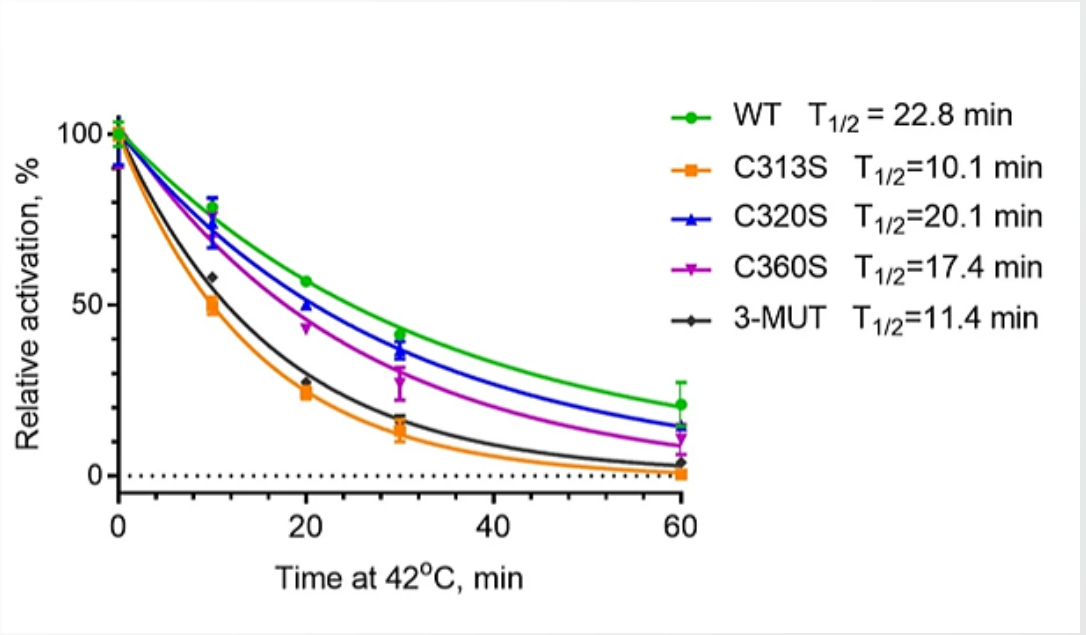
Mammalian expressed CB2 receptor has higher activity than CB2 expressed in *E. coli*

Greater CB2 thermostability with appropriate PTMs

N-glycosylation and C-term palmitoylation of CB2 receptor is critical for activity



Stability at 42 °C in Façade-TEG/CHS micelles



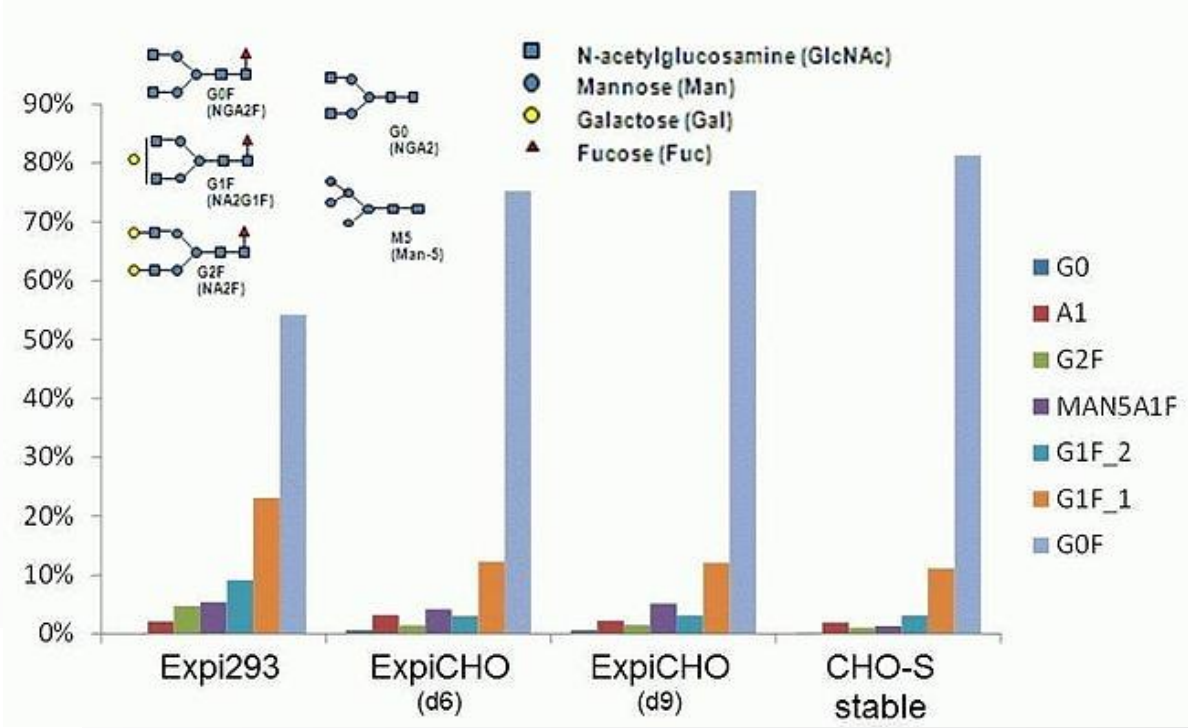
Yeliseev, A., et al. (2020) Nature Res. Sci. Reports 10:16805 | https://doi.org/10.1038/s41598-020-73813-7 | https://creativecommons.org/licenses/by/4.0/

ExpiCHO system

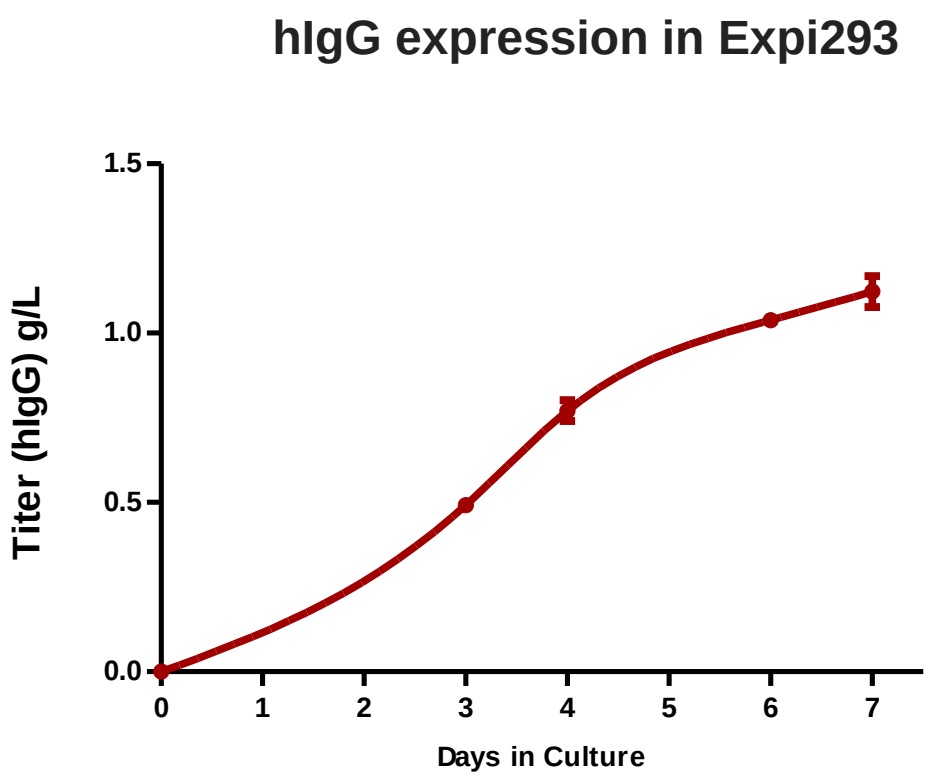
ExpiSf cell line attributes

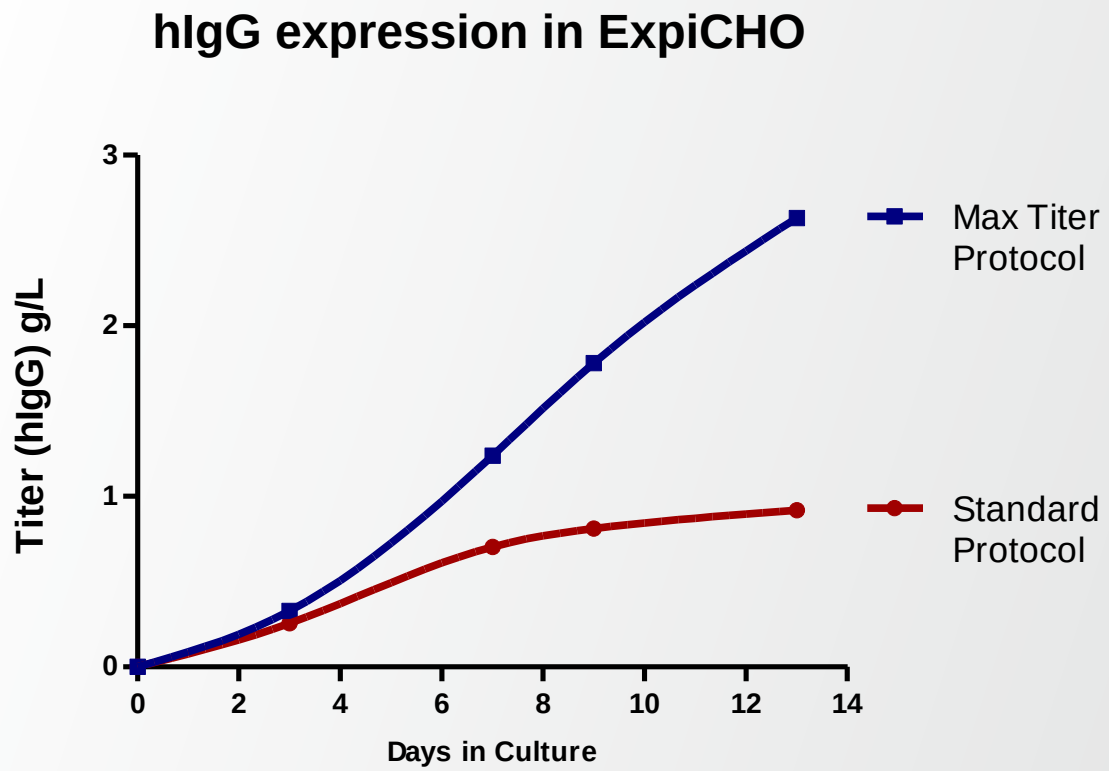
- Sub-clone derived from GMP CHO-S cells
- Adapted for high-density culture (≥20M cells/mL)
- Short doubling time (~17-18 hours)
- Cell diameter 14 - 20µm (culture – expression)
- High transfection efficiency (75-80%)
- Stable growth and expression profiles for ~20 passages
- “CHO-like” glycosylation profiles to match stable bioproduction
- High quality, biologically-active protein

- Human IgG overexpression



Expi293 vs ExpiCHO Systems: Expression kinetics



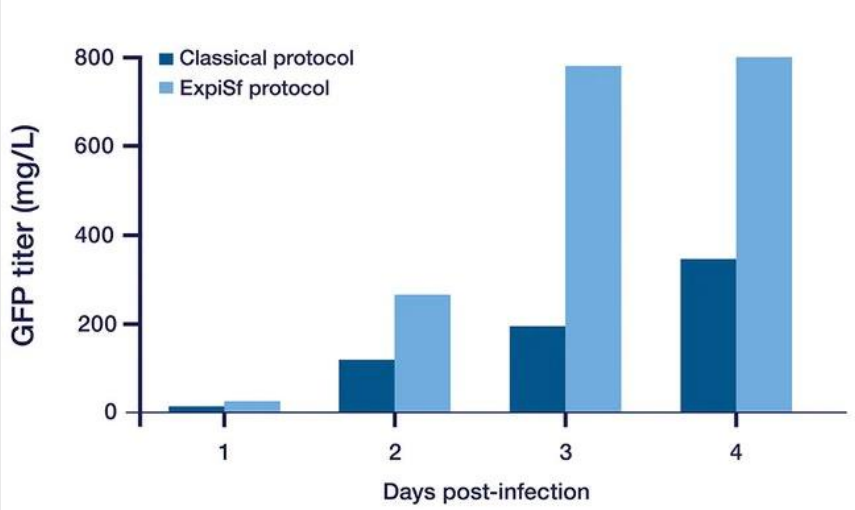


*Expi293 has fast, but short, expression profile. ExpiCHO can generate similar titers in the first 7 days, however, ExpiCHO can continue to express out to 14 days.

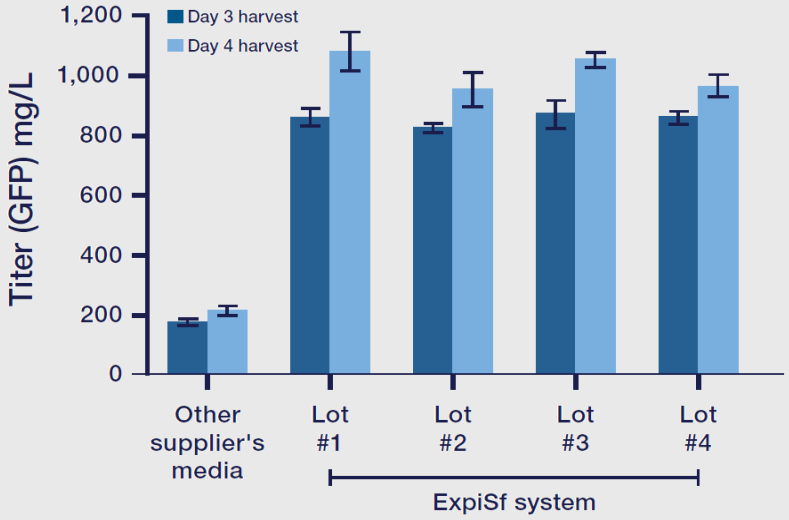
ExpiSf system

ExpiSf expression system attributes

- Achieves 3 times more protein than current insect platforms
- First-ever, chemically defined insect growth medium
- Consistency over multiple expression runs
- Optimized, fully-integrated system
- Robust production of high-titer, high-quality P0 recombinant baculovirus in suspension culture (no virus amplification needed)
- Reduced time to protein (6-10 days) compared to classical workflows (12-20 days)



Lot-to-lot consistency of ExpiSf CD medium



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4. Automated purifications with magnetic supports
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Protein extraction and enrichment reagents

- **Performance**—many first-to-market reagents with trusted performance
- **Optimized**—maximize protein yield and preserve protein activity
- **Efficient**—minimal cross-contamination between subcellular fractions
- **Compatible**—extracts can be used directly in most downstream applications
- **Gentle**—eliminate mechanical cell disruption for most sample types

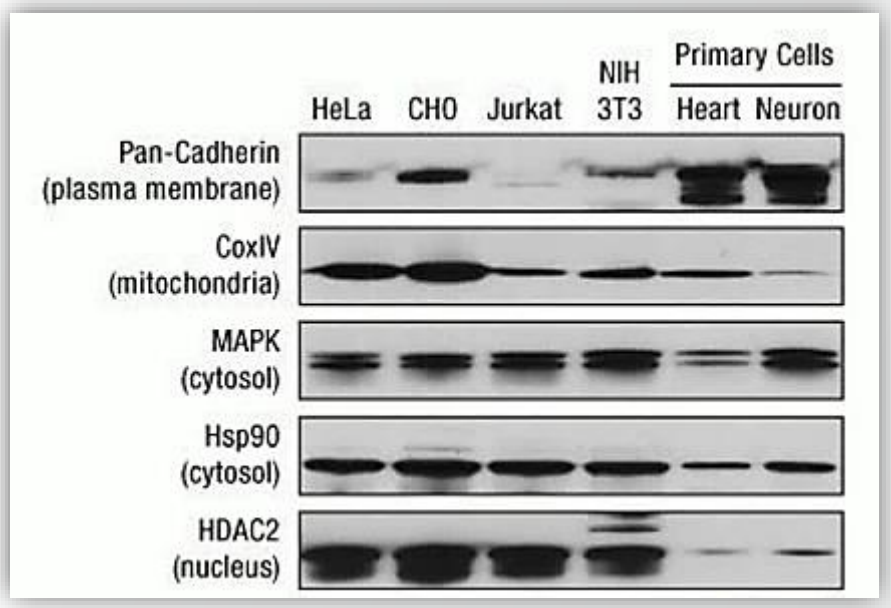
Total protein extraction reagents (PERs)		Protein fractionation kits
General lysis reagents	**Cell-specific "Pop-PERs"**	**Subcellular, organelle, and tissue specific**
• RIPA buffer • IP lysis buffer • Individual detergents	• Mammalian (M-PER) • Bacterial (B-PER) • Tissue (T-PER) • Yeast (Y-PER) • Insect (I-PER) • Plant (P-PER)	• Nuclear and cytoplasmic (NE-PER) • Membrane (Mem-PER) • Subcellular fractionation kit (cells/tissue) • Synaptosome isolation kit • Mitochondrial isolation kit

M-PER: Mammalian protein extraction reagent

	M-PER

Features	• Proprietary formulation with a **dialyzable** detergent
	• Whole-cell lysate
	  • All cell compartments lysed
	  • Helps proteins **maintain their native structure** and preserve any protein:protein interactions

Sample type	• Mammalian cultured cells
Applications	• SDS-PAGE • Western blots • Immunoprecipitations (IP / Co-IP) • Pull-downs • Activity assays
Recommended Thermo Scientific™ Pierce™ protein assay*(s)*	• BCA Protein Assay Kit • Rapid Gold BCA Protein Assay Kit • Detergent Compatible Bradford Assay Kit

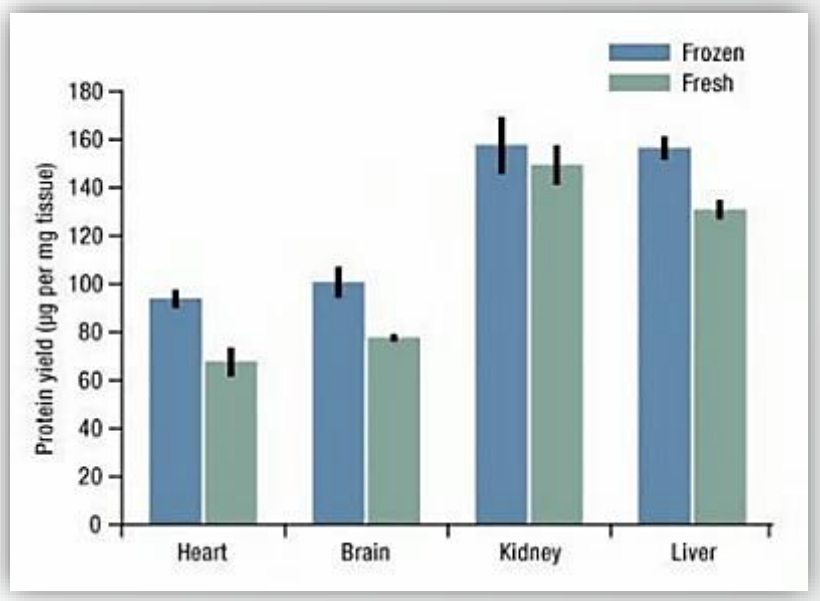


Western blot of cell lines showing M-PER liberates proteins from different cellular compartments

General lysis reagent that is useful for many applications, detergent is dialyzable

T-PER: Tissue protein extraction reagent

	T-PER
Features	• Proprietary formulation with **dialyzable** detergent • All cell compartments lysed • Helps proteins **maintain their native structure** and preserve any protein:protein interactions • Mechanical disruption required with reagent
Sample type	• Mammalian tissue *(fresh or frozen)*
Applications	• SDS-PAGE • Western blots • Immunoprecipitations (IP / Co-IP) • Pull-downs • Activity assays
Recommended Pierce protein assay(s)	• Detergent Compatible Bradford Assay Kit • BCA Protein Assay Kit (1:2) • Rapid Gold BCA Protein Assay Kit (1:2)



Protein yield comparison of T-PER with four tissue types, fresh and frozen. Fresh samples typically yield more protein.

General lysis reagent for mammalian tissue samples

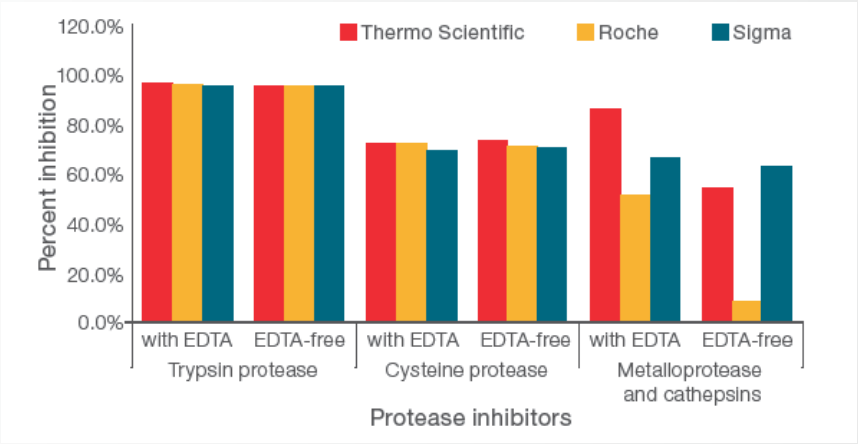
Protein preservation

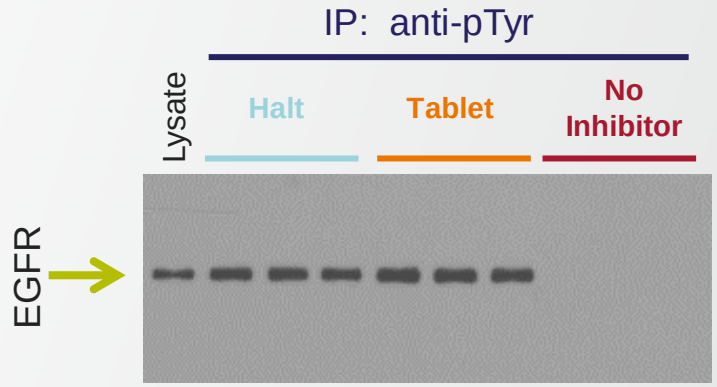
Thermo Scientific™ Halt™ and Pierce™ protease and phosphatase inhibitors

- **Multiple formats – liquid cocktails** or **fast dissolving tablets** in multiple pack sizes
- **Convenient –** ready-to-use, broad enzyme spectrum formulations for excellent protein protection
- **Combined cocktail –** available as all-in-one formulation containing **both protease and phosphatase inhibitors**
- **Consistent –** liquid and tablet have the same inhibitor concentrations in final sample (except for XL capsule)

	Halt inhibitor liquid cocktails	Pierce inhibitor tablets
Flexible addition based on sample volume?	**YES**	**NO**
Requires reconstitution?	**NO (100X)**	**YES**
Pricing	**Premium**	**Economical**

Protease tablets





Importance of phosphatase inhibitors

Outline

1 Expression systems

2 Cell / tissue extraction

3 Affinity purification (resins/beads)

4 Automated purifications with magnetic supports

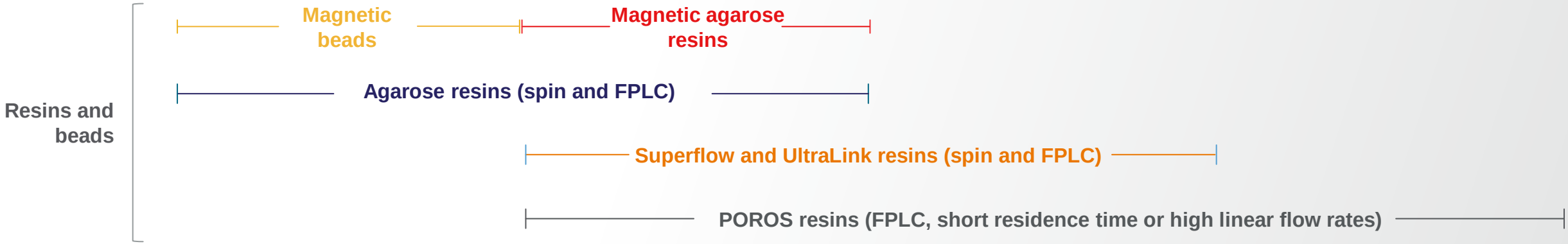
5 Protein clean-up

Scale of protein purification

Scale of purification
Protein yield: µg, mg, g, kg

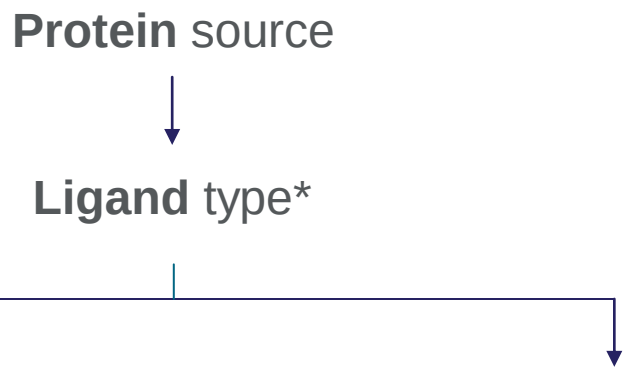
Thermo Scientific™ **Resin** types
Agarose, Superflow™ agarose, Pierce™ magnetic agarose, Pierce™ magnetic beads, Pierce™ UltraLink™ resin, and POROS™ resin

Scale	Micro	Batch	Pilot	Process
Yield	ng–mg	mg–g	g–kg	kg–more



Superflow is a trademark of Sterogene Bioseparations, Inc.

Ligand-based protein purification



Ion exchange	Affinity			
Native protein charge	Antibody	Recombinant-tag	Biotin-tag	Thermo Scientific™ custom ligands
(+) Strong cation exchange (–) Strong anion exchange	Protein A Protein G Protein A/G Protein L	Ni-NTA or Cobalt (His-tag) Glutathione (GST-tag) Anti-HA (HA-tag) Anti-c-myc (c-myc-tag) Anti-DYKD$_4$K (FLAG-tag)	Avidin Monomeric avidin Streptavidin NeutrAvidin	SulfoLink™ ligand AminoLink™ ligand CarboxyLink™ ligand GlycoLink™ ligand NHS-activated ligand

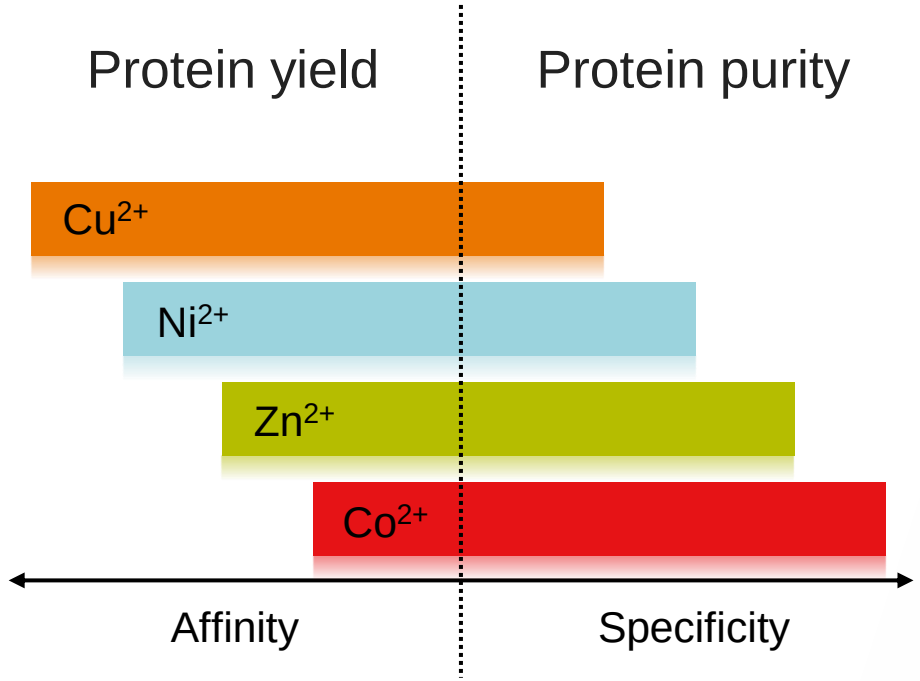
* Not all ligands are available in all support types.

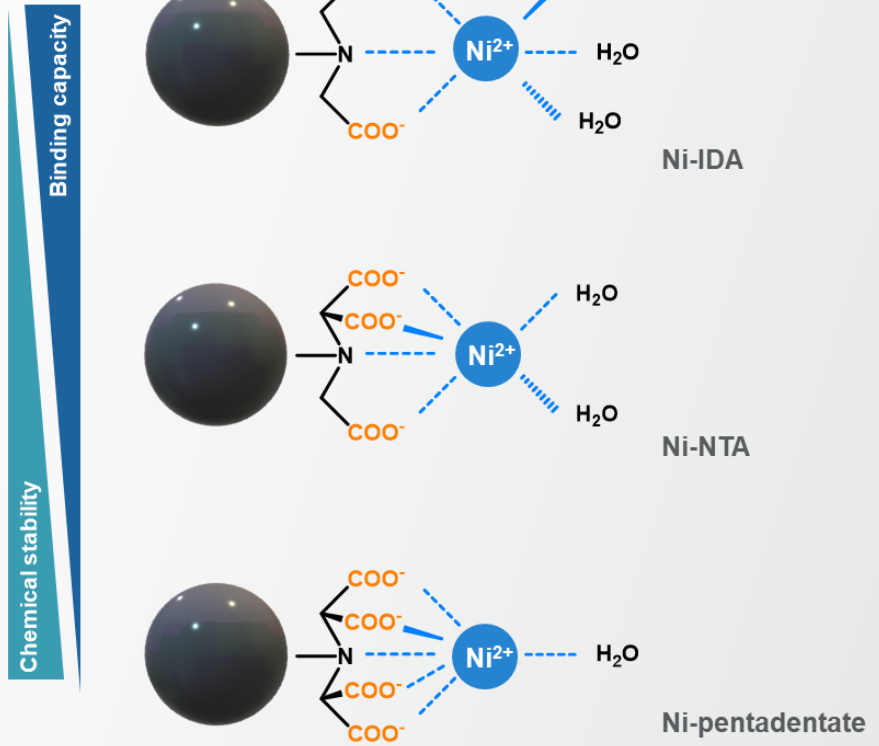
What epitope tag should I use?

Epitope tag	Size / identity	Ligand	Affinity (K_D)	Elution	Advantages	Challenges
IMAC	6xHis (can vary from 4–16 histidines)	Metal-chelate (Ni or Co)	~1 mM	• Imidazole	• Small tag minimizes impact on structure/function • Can purify under denaturing conditions • Easy to use • Low cost • Can regenerate	• Concerns with Ni leach • Purity is variable
GST	224 amino acids (26 kDa)	Glutathione (reduced)	~110 nM	• GSH	• Can improve solubility • Important for high level expression in prokaryotes • Low cost	• Larger tag may interfere with structure or function
Small peptide	HA (YPYDVPDYA) c-Myc (EQKLISEEDL) FLAG (DYKDDDDK) (1x and 3x variants)	Anti-HA Anti-c-myc Anti-FLAG	pM to low nM	• Acidic buffer • Competing peptide	• Achieves higher purity, minimize 2º cleanup • Good for IPs • Small tag minimizes impact on structure/function • FLAG has built-in enterokinase cleavage site	• More expensive antibody supports • Re-use is limited

Choices of metal ions for His-tag purifications

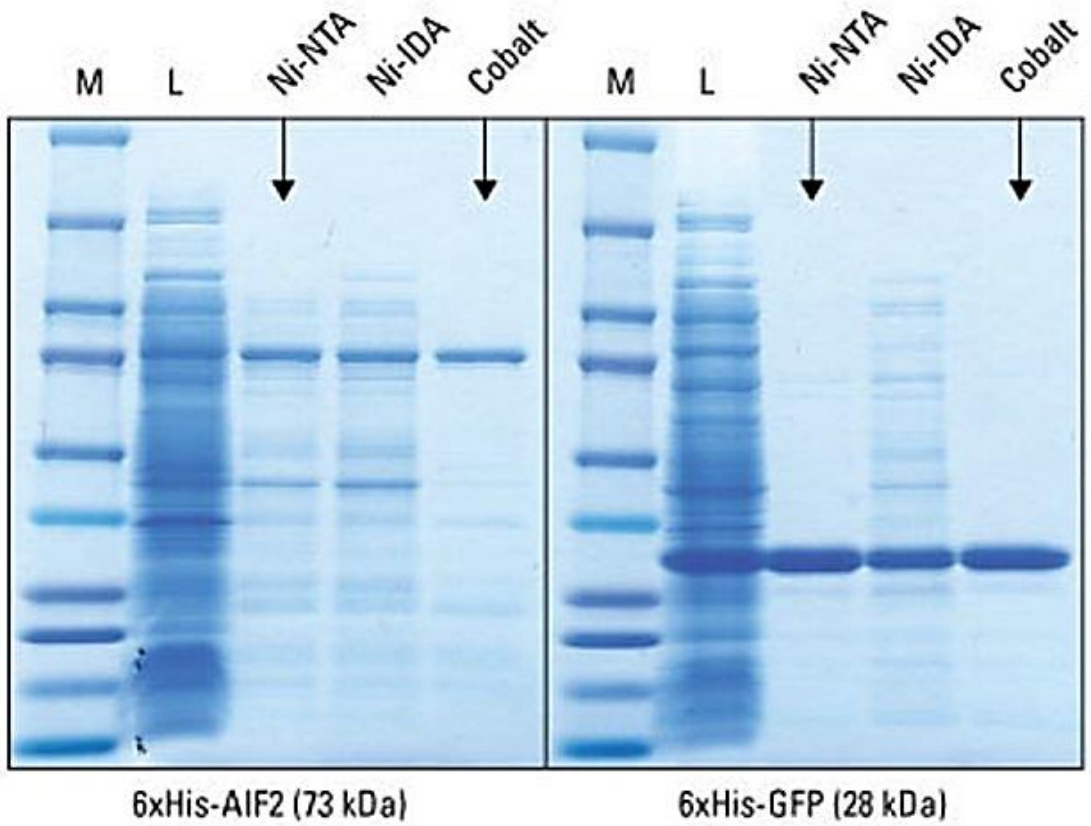
Many IMAC versions to choose from…

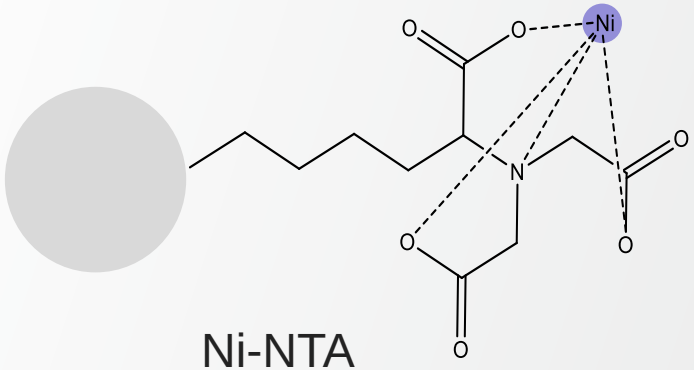




6XHis-protein purity dependencies

Expression level, chelator, metal used





Ni-NTA

	6xHis AIF2 (73kDa)		6xHis GFP (28kDa)	
Resin	**Yield**	**Purity**	**Yield**	**Purity**
HisPur Ni-NTA	0.5mg	32%	0.8mg	90%
Ni-IDA	0.5mg	25%	0.6mg	52%
HisPur Cobalt	0.4mg	49%	0.7mg	91%

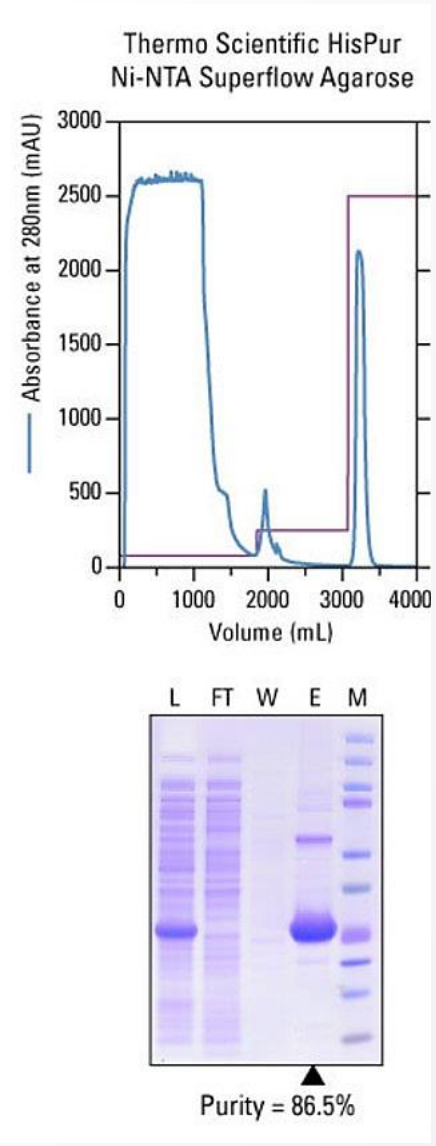
Ni-NTA most popular, has higher capacity. Cobalt-IMAC gives higher purity, best for low expressers.

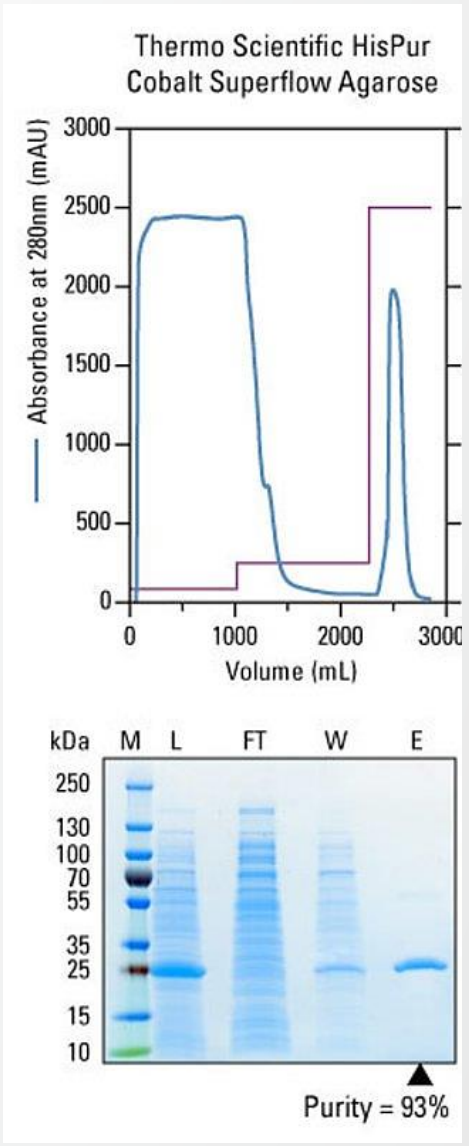
Ni for higher capacity; Co for higher purity

Dynamic binding, FPLC format, SuperFlow resins

High-yield, high-purity, medium-scale purification of 6xHisTagged protein

More than 4 grams of over-expressed 6xHis-GFP were purified in 3 hours using 200mL columns containing HisPur Ni-NTA Superflow Agarose or Qiagen™ Ni-NTA Superflow. One liter of lysate was loaded at a flow rate of 20mL/min, then washed until baseline with wash buffer containing 30mM imidazole. Bound protein was eluted with buffer containing 300mM imidazole. Fractions containing purified 6xHis-GFP were pooled and quantitated using Pierce 660nm Protein Assay (Part No. 22662). Load, flow-through, wash, and eluate fractions were separated by SDS-PAGE, stained with Imperial Protein Stain (Part No. 24615) and evaluated using myImageAnalysis Software (Part No. 62237) to determine purity.





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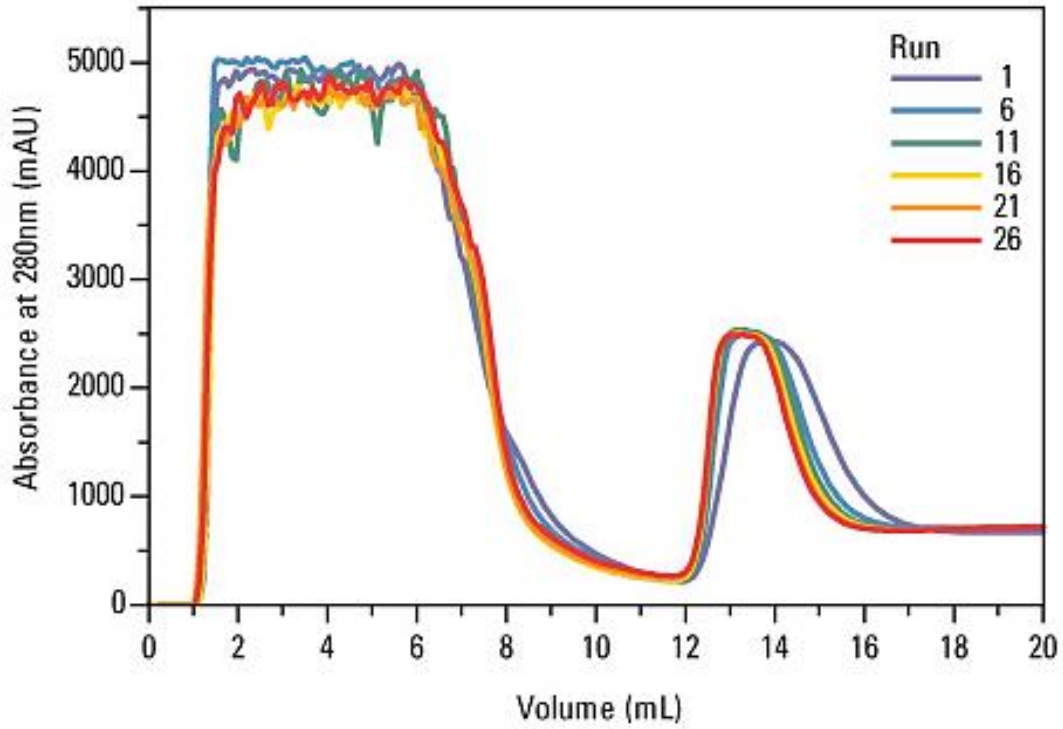
Re-use of Ni-NTA Superflow agarose



Column: 1mL HisPur Ni-NTA Superflow agarose

Sample: 6XHis-GFP in *E. coli* lysate

Regeneration / Clean-in-Place protocol:

- 10 vol 0.5M NaOH
- 10 vol dH_2O
- 10 vol binding buffer

Optional regeneration protocol:

- *EDTA strip, re-charge with Ni_2SO_4*

Ni-NTA resin can be CIP'ed and reused at least 25X with no loss of performance

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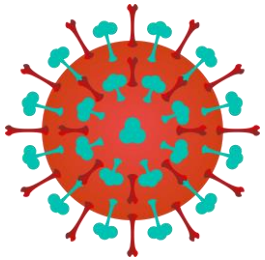
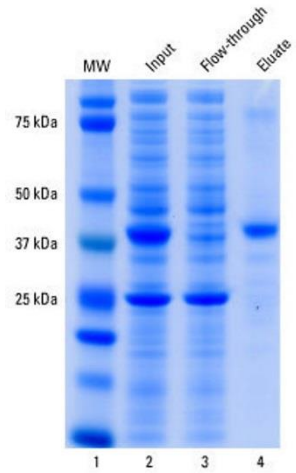
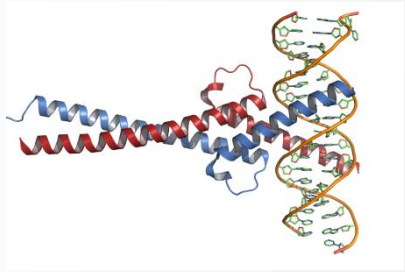
Immobilized anti-epitope tag purification supports

- **HA-tag (YPYDVPDYA)**
  - High affinity MAb identified to bind to human influenza hemagglutinin
  - Epitope mapped to aa 98-106







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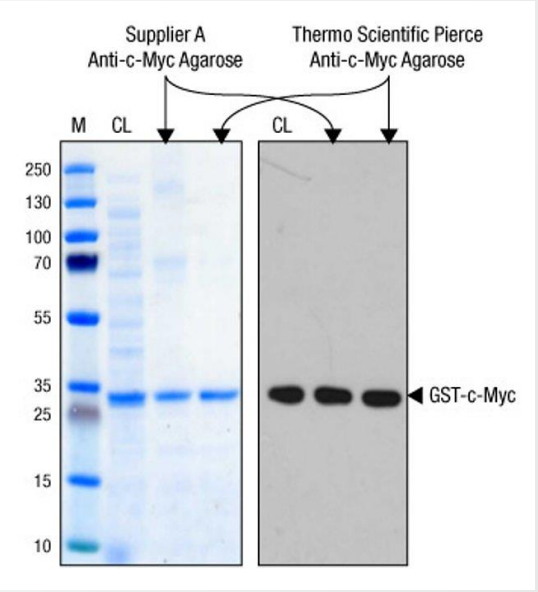


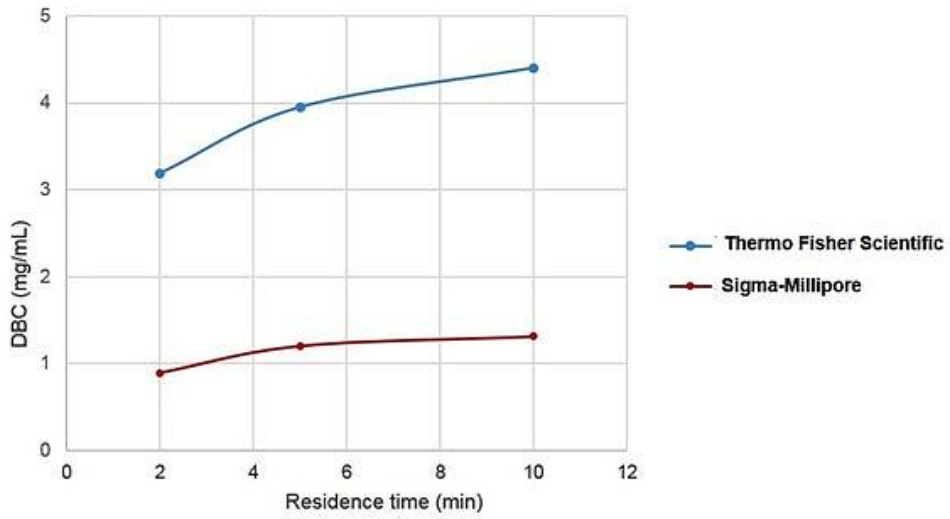
- **c-Myc tag (EQKLISEEDL)**
  - High affinity Mab identified that binds to c-myc gene product

- **FLAG™-tag (DYKDDDDK)**
  - Completely artificial design
  - Hydrophilic, minimize chance of inactivating fusion protein
  - MAb raised against sequence
  - N-term tag sequence cleaved by enterokinase

FLAG is a registered trademark of Sigma-Aldrich Co. LLC

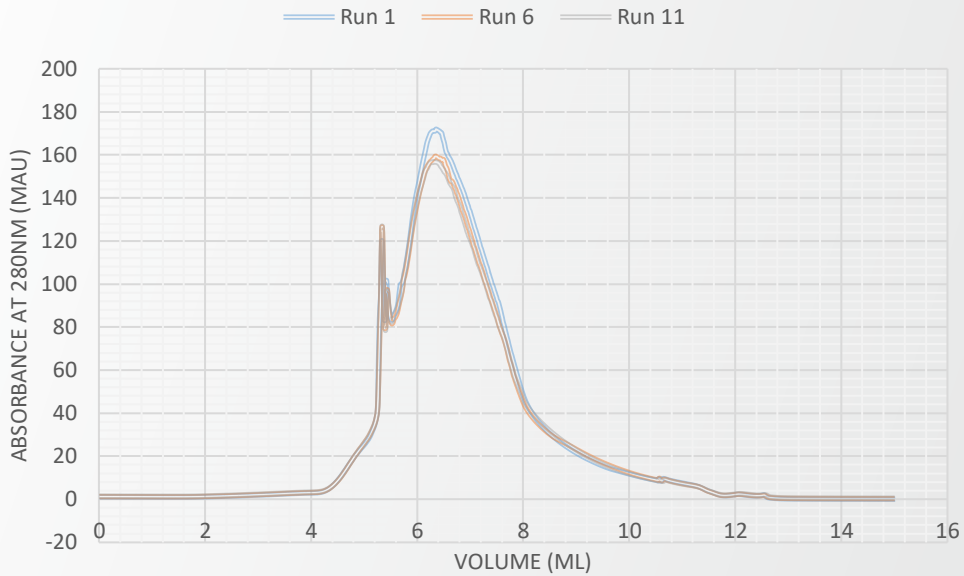
Anti-DYKDDDDK Affinity Resin

- High dynamic binding capacity
- Regeneration (10X) of the resin is possible w/o loss of function



Item	Details
Column	0.5 cmD x 5cmL (1mL)
Loading Buffer	100mM phosphate, 150mM NaCl, pH 7.2 (PBS)
Detection	UV at 280 nm
Sample	DYKDDDDK-GFP-His (1mg/mL)
DBC	10% breakthrough
Linear Flow Rates	150cm/hr, 60 cm/hr, and 30 cm/hr

Anti-DYKDDDDK Ultralink resin regeneration



1mL of settled Thermo Scientific™ Pierce™ Anti-DYKDDDDK Affinity Resin was packed into a 1mL column. It was then loaded with 4mL purified DYKDDDDK-TurboGFP-His at a concentration of 1mg/mL at a rate of 0.2mL/min. The column was subsequently washed (PBS), stripped (0.1M glycine, pH 2.8), and regenerated up to 10 times with minimal loss in binding.

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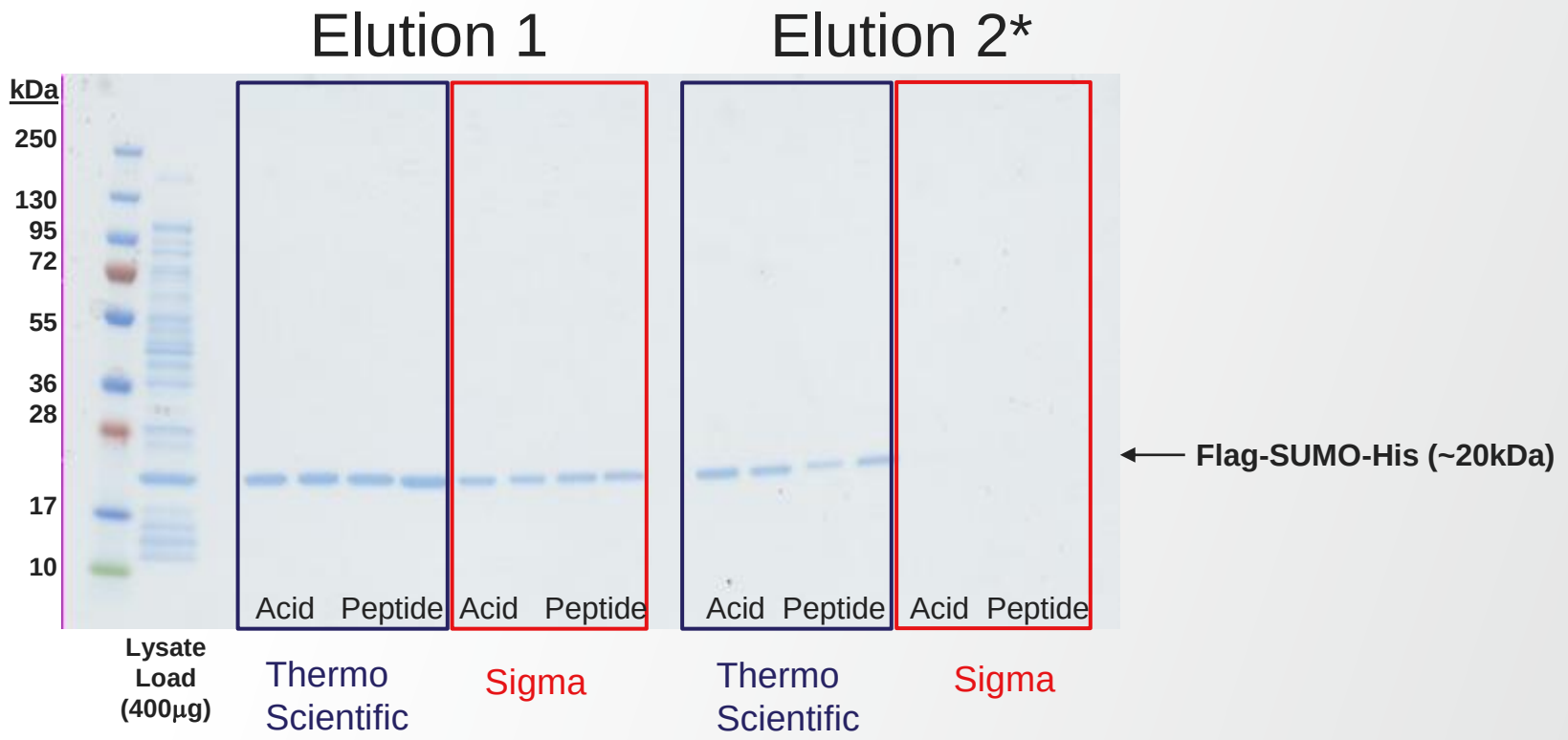
Anti-DYKDDDDK Affinity Resin

Efficient peptide and acid elution

- Higher affinity interaction
- Higher purity
- Multiple elution options:
  - 0.1M Glycine, pH 2.8
  - 3X DYKDDDK peptide



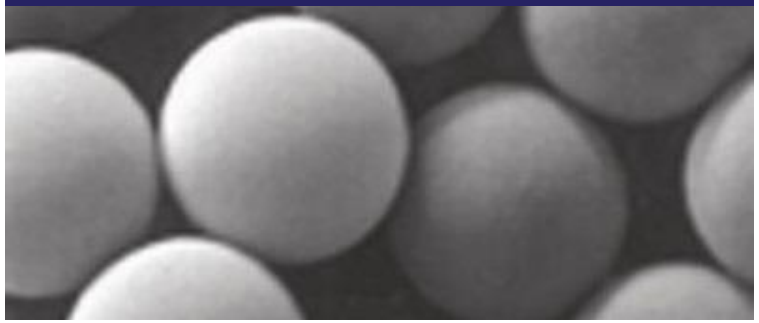
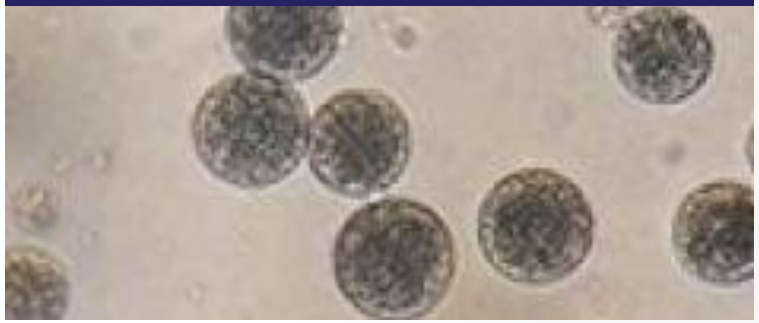
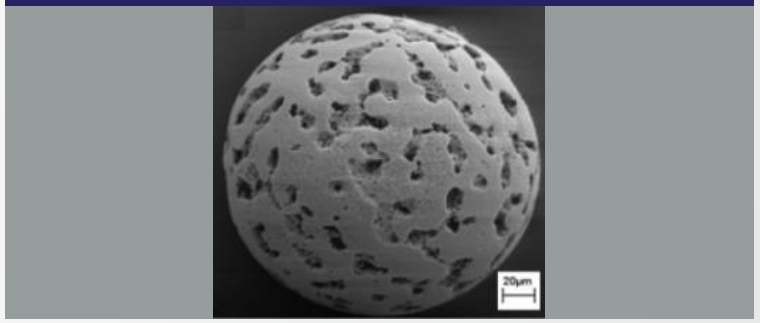
Sample: bacterial overexpression lysate

Outline

1. Expression systems
2. Cell / tissue extraction
3. Affinity purification (resins/beads)
4. Automated purifications with magnetic supports
5. Protein clean-up

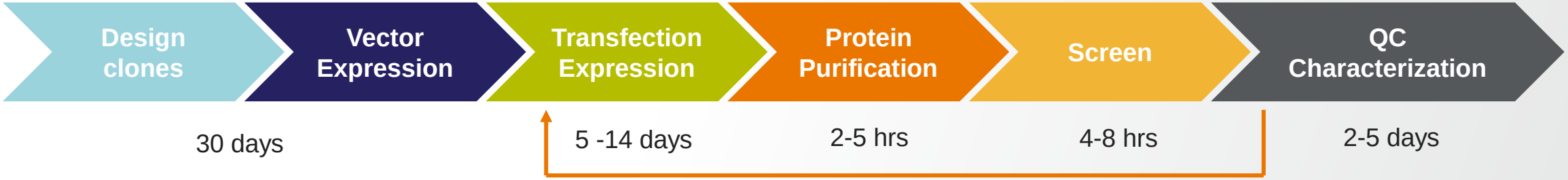
Magnetic beads for protein purification

IP and pull-downs → **Protein purification**

ng–µg	mg–g	g–kg
Magnetic beads	**Magnetic agarose**	**Non-magnetic resins**
		
• Automated, HTP apps	• Automated, HTP apps • Higher binding capacity • Screening	• >g-scale purification • Highest binding capacity
Pierce and Dynabeads magnetic beads	Thermo Scientific™ Pierce™ magnetic agarose	Agarose, UltraLink, and POROS resins

Advantage of high-capacity screens

- **Need for optimized and connected workflows (expression→ purification→ analysis)**
- **Automated, mg-scale protein purification solution for screening and characterization**
  - Purification of overexpressed proteins from cell culture media
  - Need for efficient, inexpensive and high purity affinity solutions
  - Need to balance investment of time with workflow optimization and other delivery goals



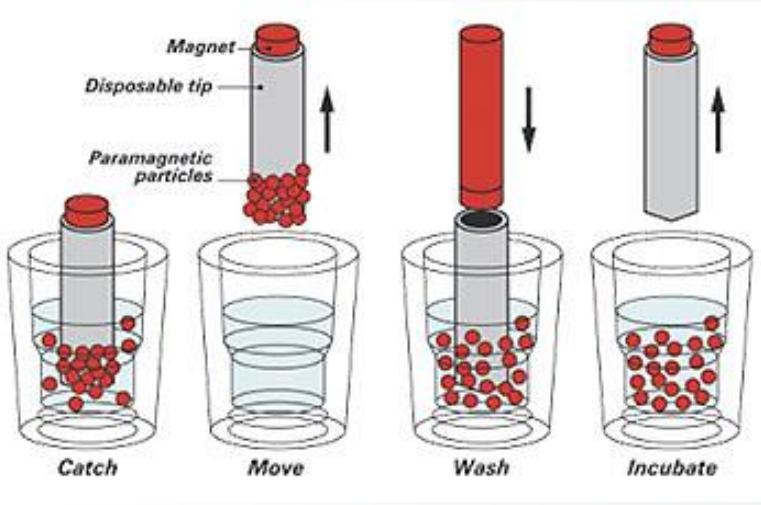
Purification with high capacity MagBeads enables proceeding directly into characterization
Time Savings: 2-4 weeks

Automating purification

Thermo Scientific™ KingFisher Flex Purification System

Protocol:

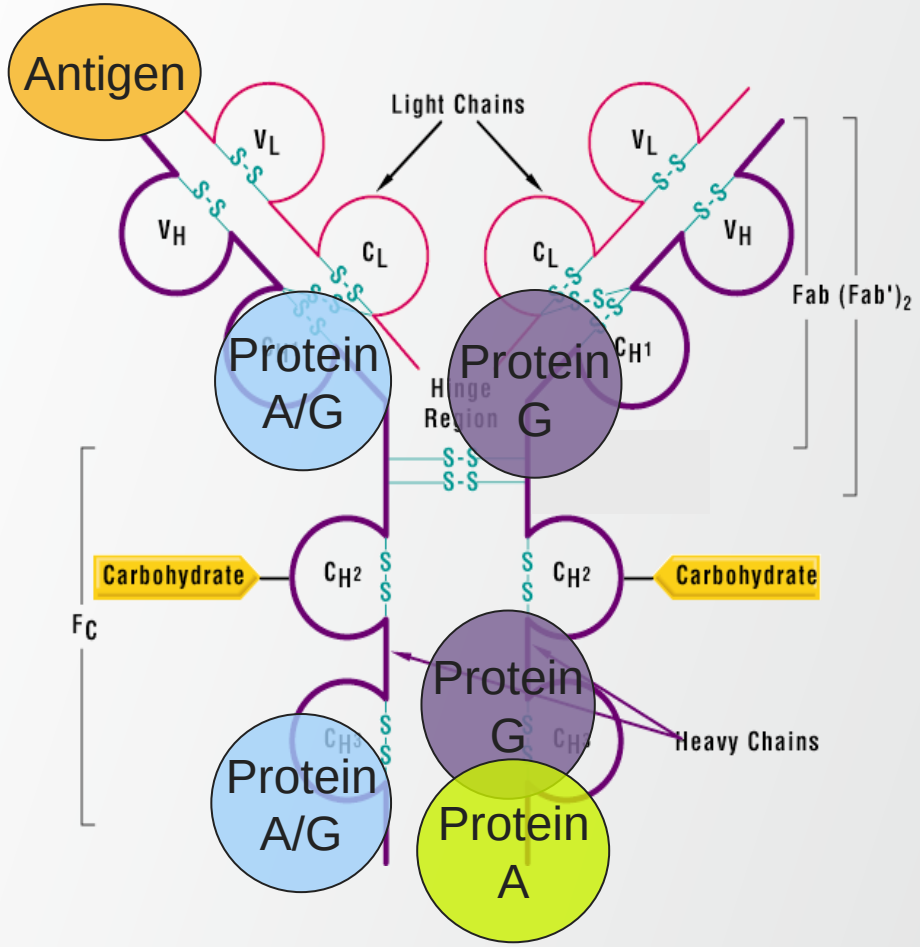
1. **Bind** 0.5mL ExpiCHO Sup + Protein AG magnetic agarose (50mL settled beads)
2. **Wash** 2 x 30 sec with 500mL PBS
3. **Wash** 30 sec with 500mL Water
4. **Elute** 5 min with 200mL 0.1M Glycine, pH 2.8



Selecting an IgG binding support

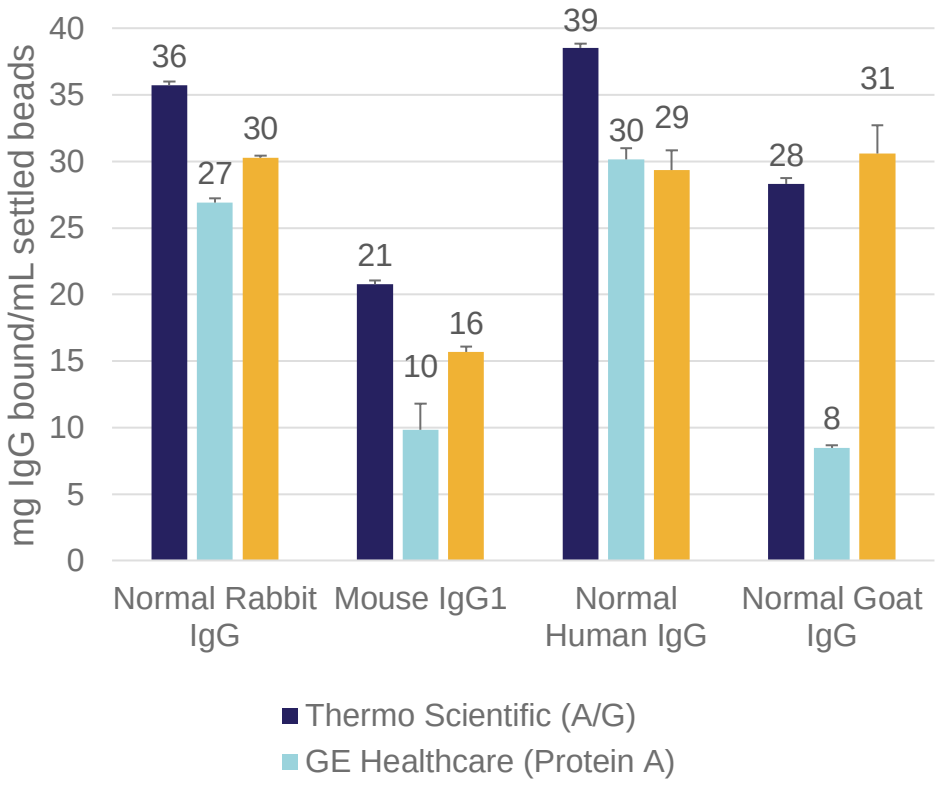
Protein A and Protein G have different affinities for antibody species and subtypes

- Protein A
  - 85% of market – chemical tolerance, easier elution
  - Binds Fc region at C_H2-C_H3 sites primarily and weakly to V_H3 (some FAbs)
  - Poor binding to IgG_3 and rat, goat antibodies
- Protein G
  - Binds Fc region and C_H1 region of light chains (purifies all FAbs)
  - Good for purifying mouse monoclonals (binds all subtypes)
  - Poor binding to Ig subtypes (e.g., IgA, IgM, IgE, etc.)
- Protein A/G
  - Engineered protein combining four Protein A and two Protein G antibody binding sites; removal of albumin binding site
  - Binds all species and subtypes that Protein A and G bind individually
  - A one-resin-fits-all solution

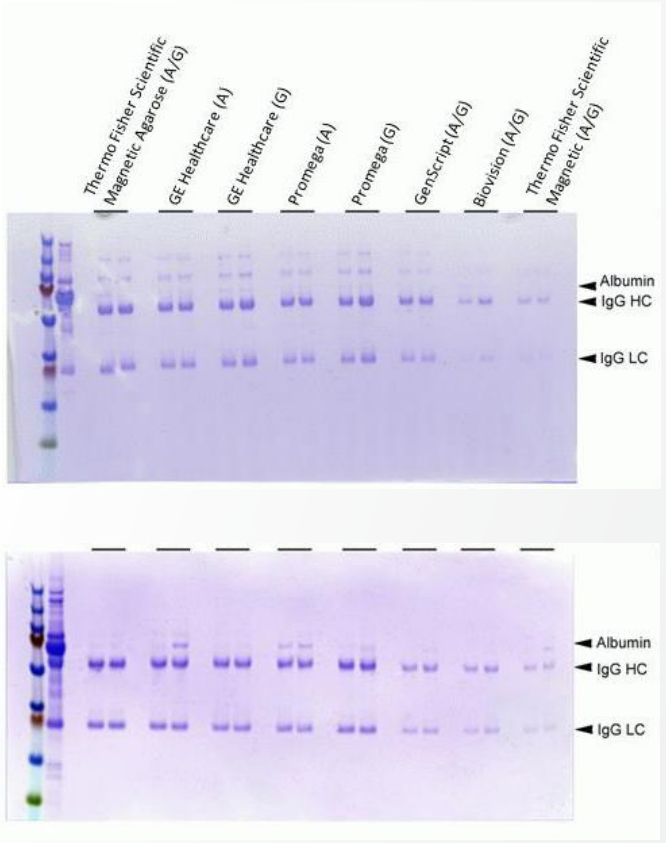


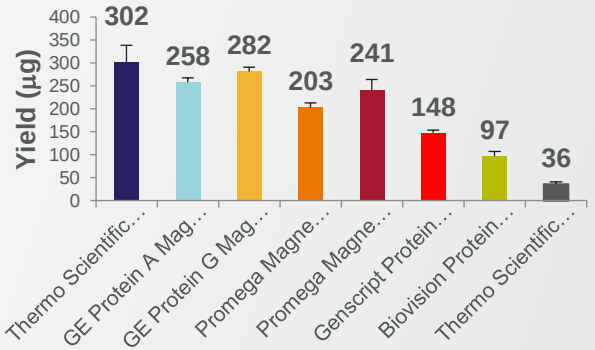
High-throughput antibody purifications and screens

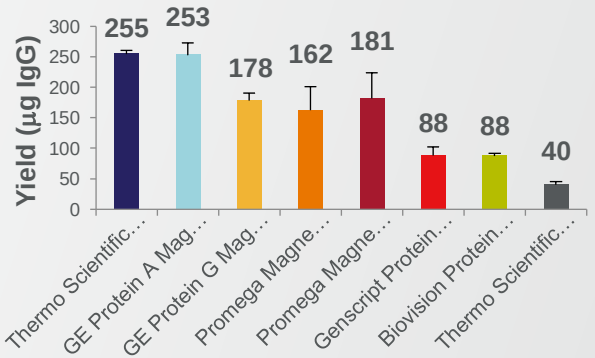
High capacity across IgG isotypes



Increased yield of IgG purified from serum





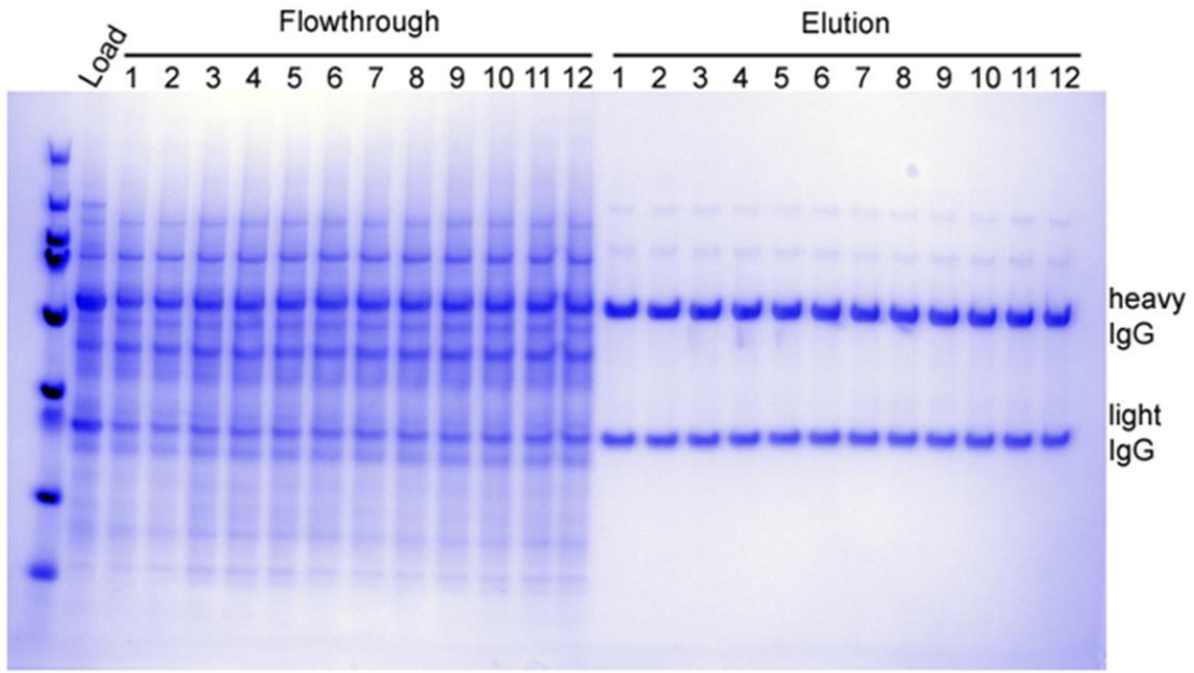


Versatility of using Pierce Protein AG Magnetic Agarose

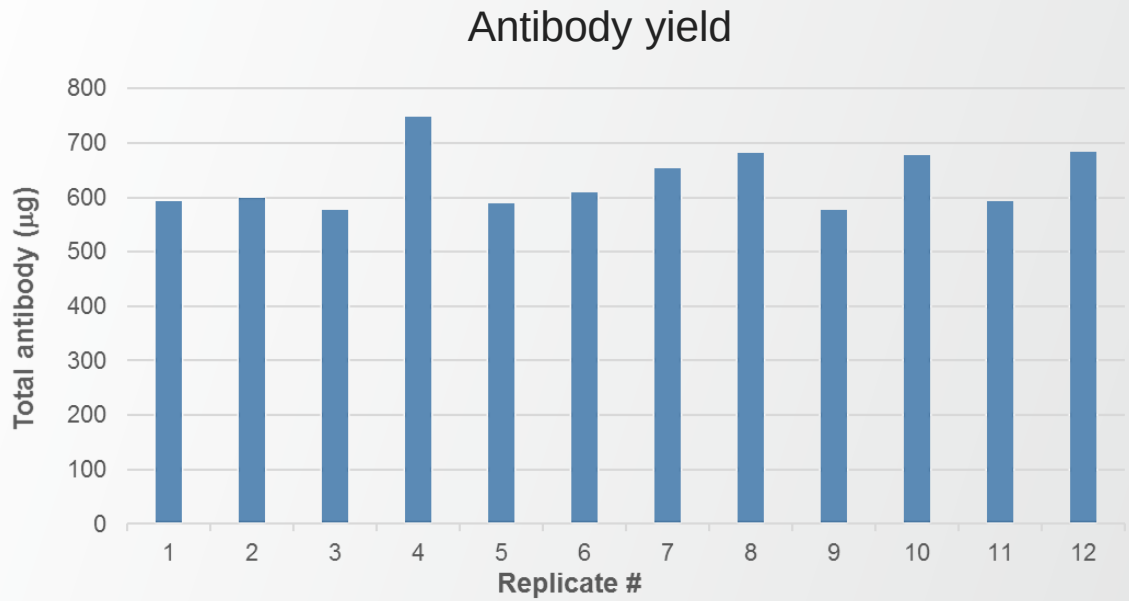
Automated antibody purification from ExpiCHO media

Thermo Scientific™ Pierce™ Protein A/G Magnetic Agarose Beads

A.



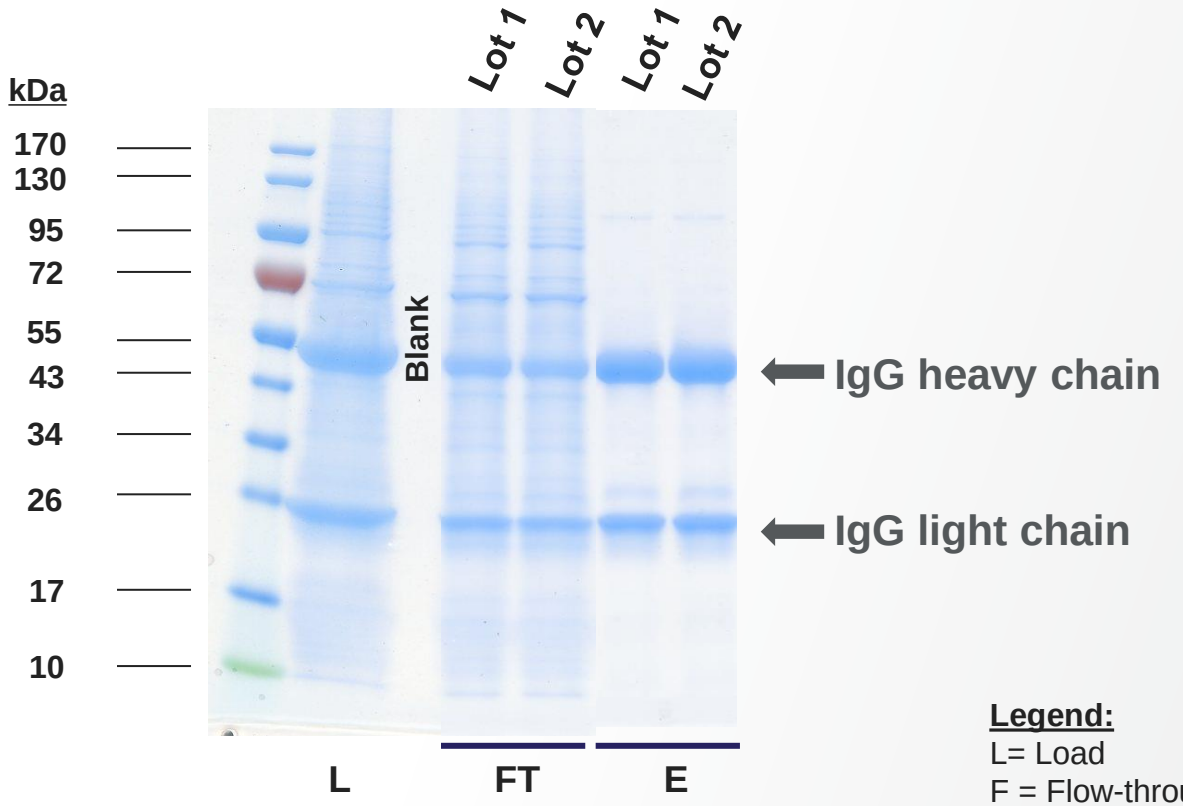
B.



ExpiCHO media expressing humanized IgG (0.5 mL) was purified with Protein AG Magnetic Agarose Beads (0.1 mL slurry) using an automated KingFisher Duo protocol. Load, flow-through and elution fractions were evaluated by reducing SDS-PAGE (panel A) to assess purity and binding efficiency. Total yield was estimated using the Detergent Compatible Bradford Assay and bovine gamma globulin as a standard (panel B). **Average yield was 632 ± 55 µg per 0.5mL sample (8.8% CV).**

Purification of Humira® Ab from Expi293 culture media

Protein A/G Magnetic Agarose Beads



Legend:
L= Load
F = Flow-through (unbound)
E = Elution

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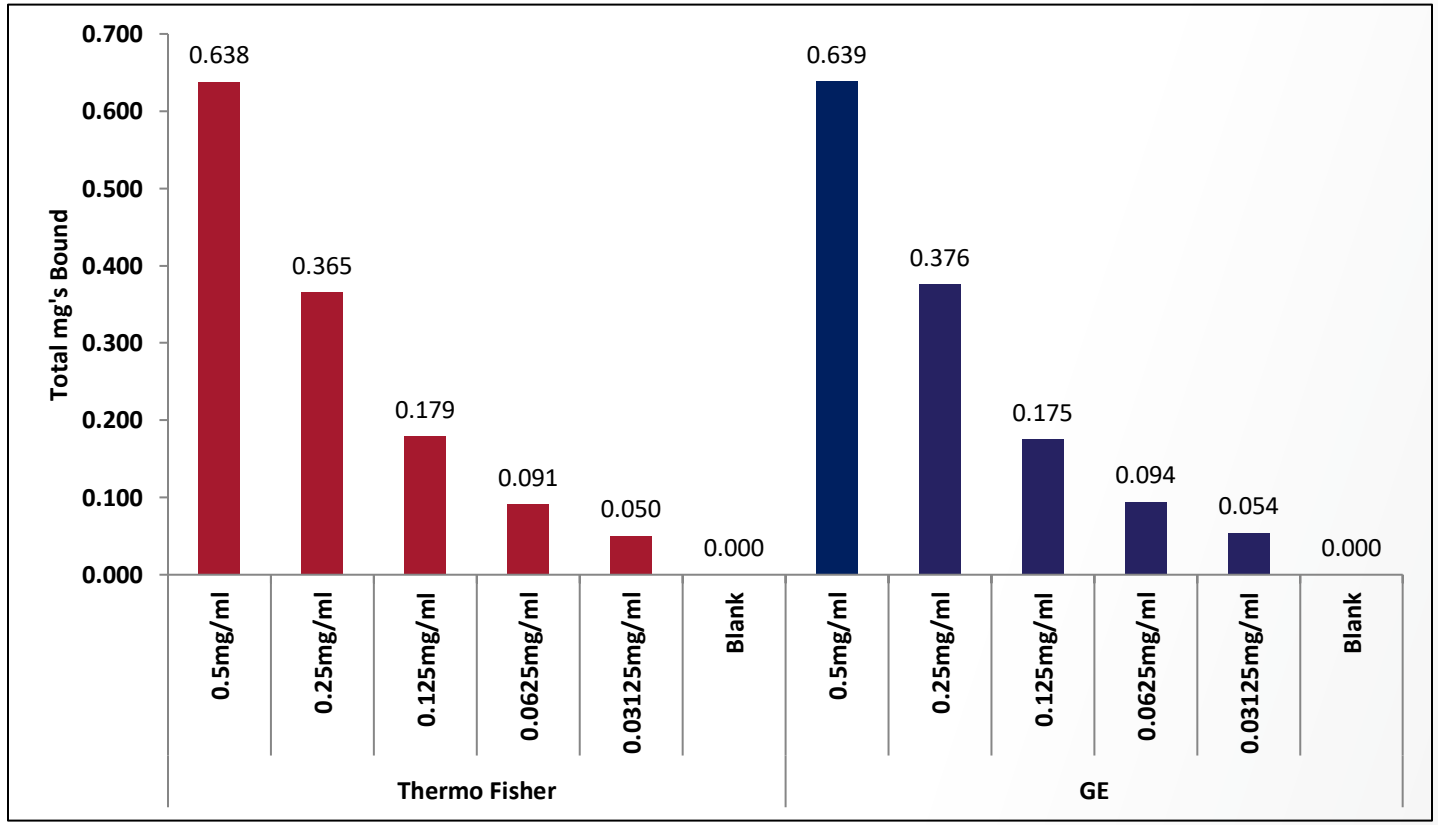
Humira is a registered trademark of AbbVie Inc.

Antibody expressed in ExpiCHO system

Mimic lower expression conditions

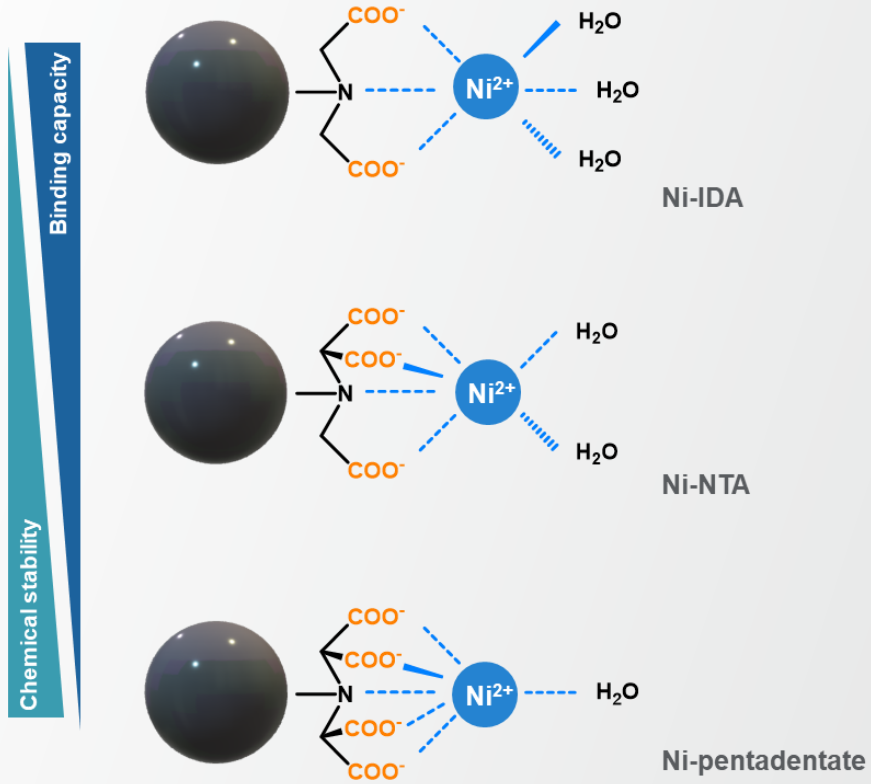


- Ab expressed in ExpiCHO media was serially diluted with blank or spent media
- 300µL of each dilution was incubated with Thermo Scientific Protein AG magnetic agarose or GE Protein A HP Spin Plate per manufacturers' protocols
- Bound antibody was washed then eluted with IgG Elution Buffer pH 2.0
- Elution fractions were collected, and protein concentration determined by BCA

EDTA-compatible IMAC supports

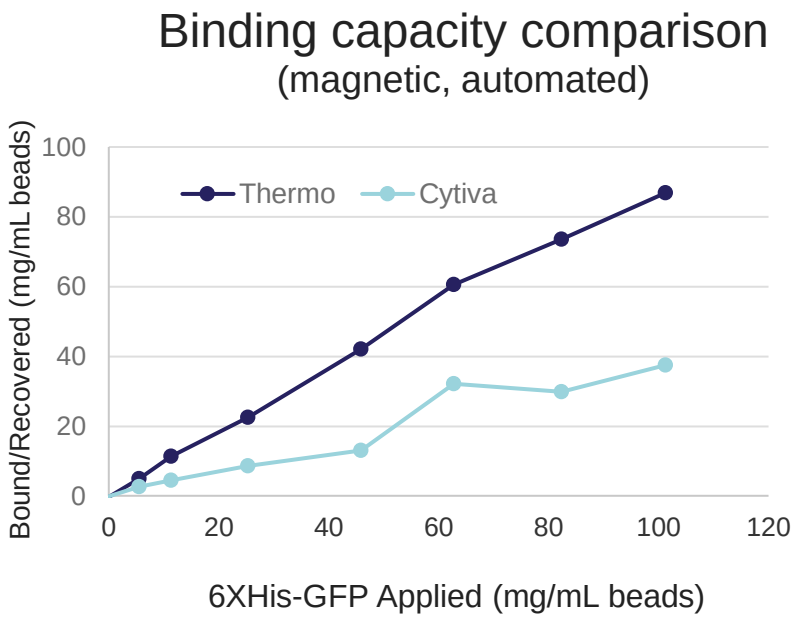
For purification of His-tagged proteins from cell culture media

- Pentadentate chelators or multi-chelate clusters
- Retain coordination with M^{2+} ions in presence of:
  - EDTA (often used as metalloprotease inhibitor)
  - Reducing agents (e.g. DTT)
  - Cell culture media / metabolites
- Critical for purifying overexpressed His-tagged proteins:
  - Secreted into the culture media
  - Sensitive proteins in lysates containing DTT and EDTA

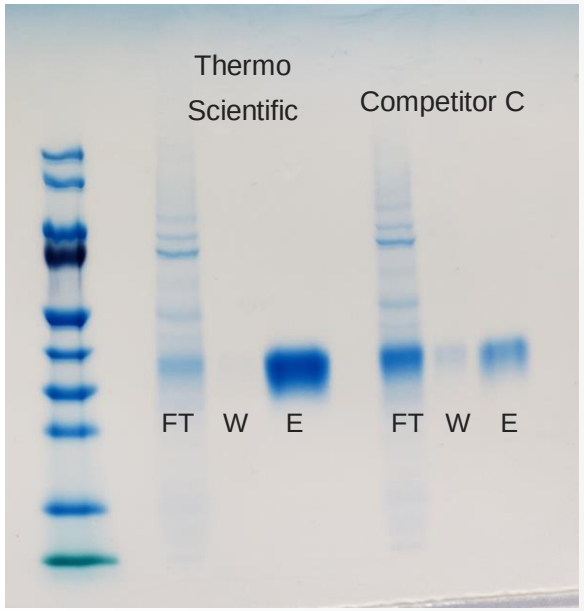


His-tagged purifications from Expi293 media

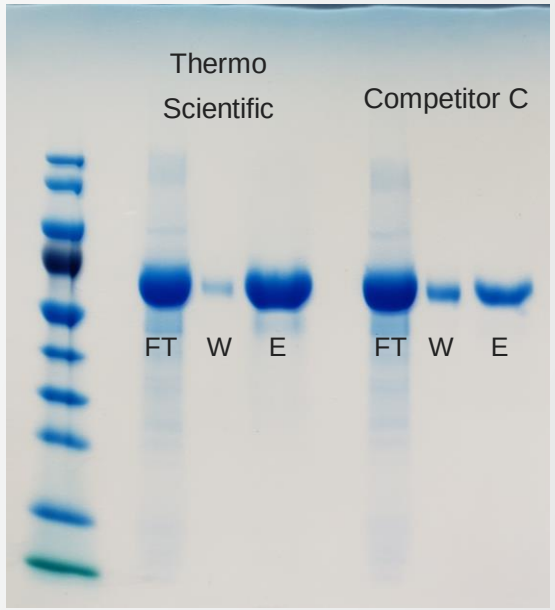
ThermoFisher™ Pierce™ High Capacity EDTA Compatible Ni-IMAC MagBeads



EPO purification



HSA purification

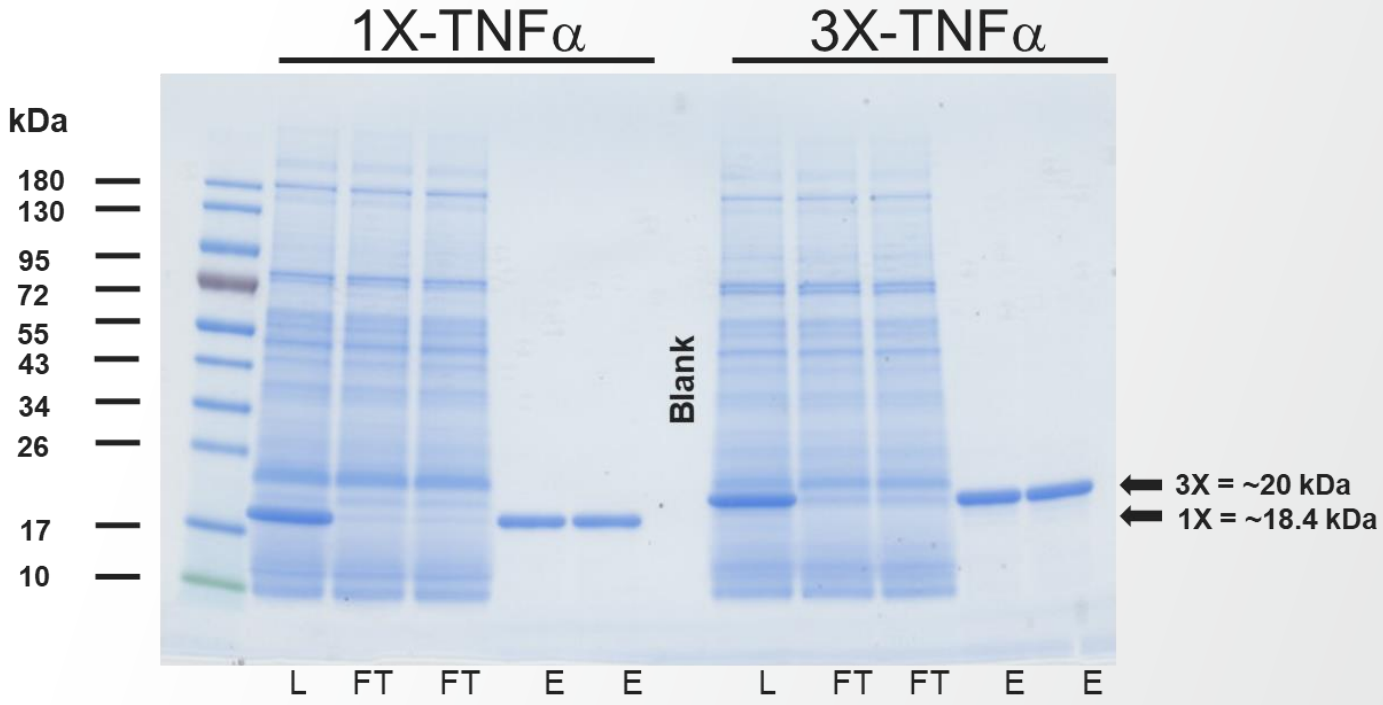


EDTA-compatible Ni-IMAC magnetic beads and resin coming soon in Q1 2021

Magnetic purification of DYKDDDDK-tagged TNFα

Both single- and triple-tagged constructs bind and elute efficiently

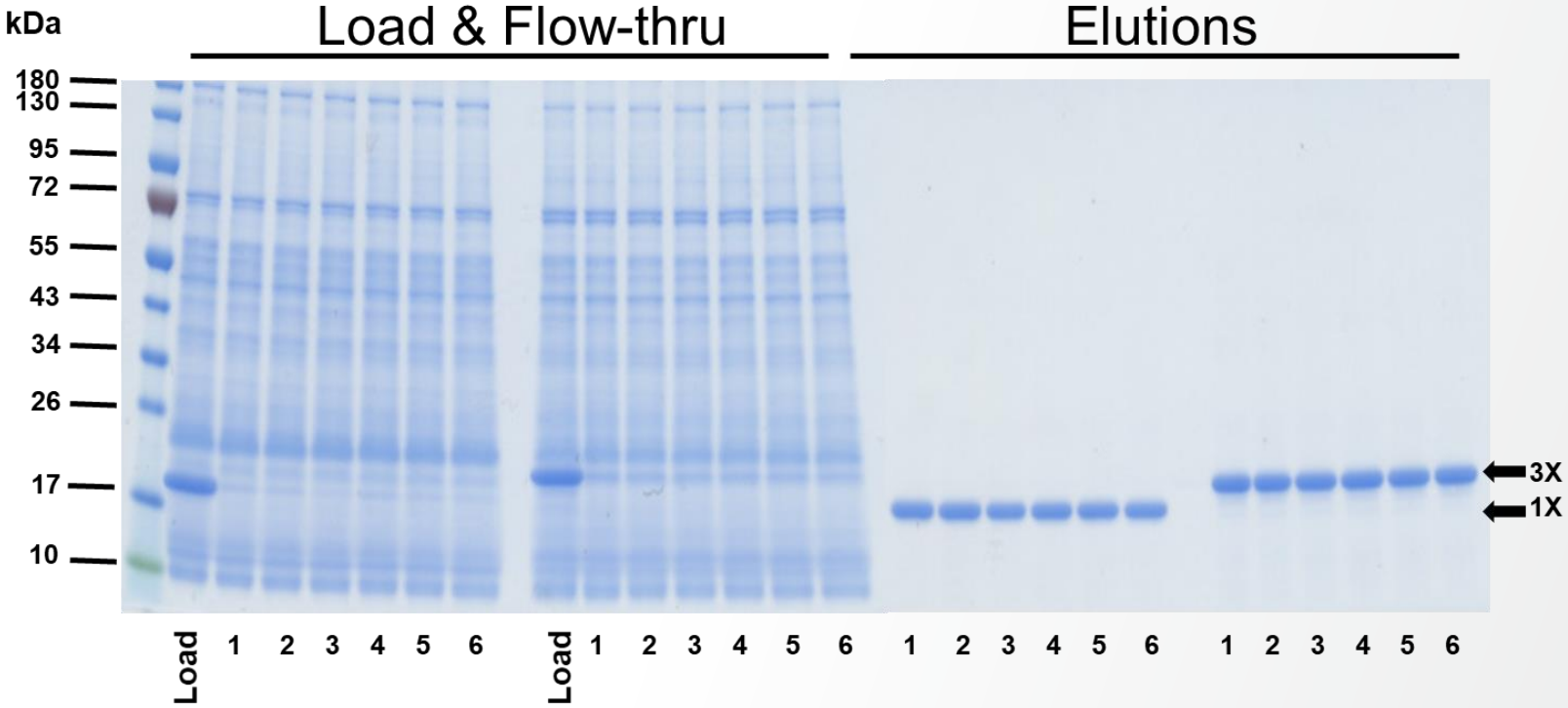
- 0.4 mL of ExpiCHO supernatant containing Flag-tagged TNFα constructs
- Anti-DYKDDDDK Magnetic Agarose (50µl settled beads)



Protein	Purified protein (µg per 0.4mL sup)	Purified protein (µg per 1mL sup)
1X-TNFα	85.6	214
3X-TNFα	142.4	356

Reproducible automated purifications

With anti-DYKDDDDK magnetic agarose

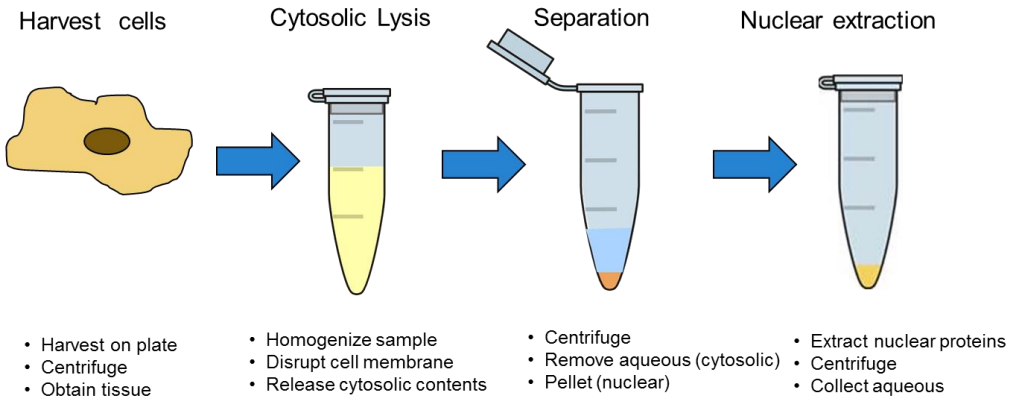


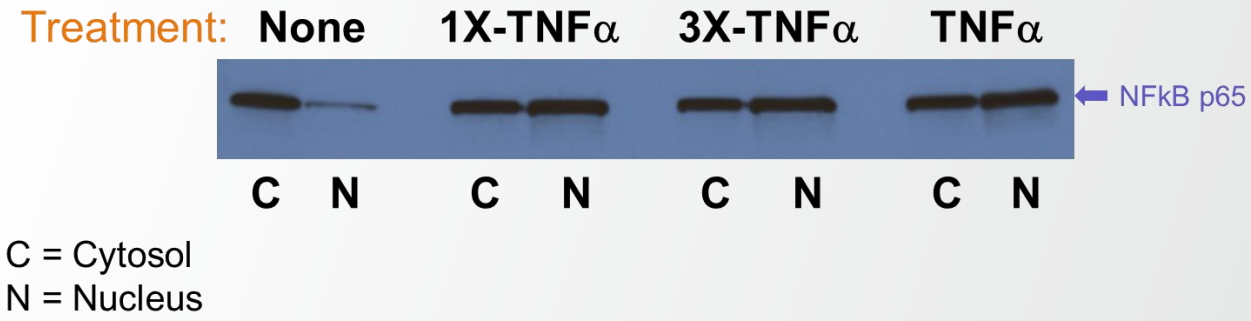
Target	Ave amount purified (mg)	Std Dev
1X-TNFa	110.8	2.8
3X-TNFa	161.8	1.5

Cytosol to nuclear translocation of TNFα

FLAG-tagged TNFα activity is preserved throughout purification

NE-PER protocol:





NFκB translocates into the nucleus with TNFα treatment, with and without FLAG tag

Positioning of protein purification supports

Protein Yield	ng - ug	mg - g	g - Kg	Kg and more
	Mag Beads	Mag. Agarose Resins		
	Agarose Resin			
		Superflow and UltraLink Resins		
		Poros Resins (FPLC Only, low residence time: 1 to 4 mins)		
Applications	• IP / Co-IP, Pull-downs • Functional studies	• Batch purification • HT purification • Structural studies	• Core Expression facilities	• Ab manufacturers • Bioprocess accounts
Formats	• Mag separation stand • Automated Processor • Microcentrifuge • Spin columns • 96-well filter plates	• Mag separation stand • Automated Processor • Spin columns • 96-well filter plates • Microcentrifuge • FPLC	• Spin columns • FPLC	• FPLC

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Outline

1 Expression systems

2 Cell / tissue extraction

3 Affinity purification (resins/beads)

4 Automated purifications with magnetic supports

5 Protein clean-up

Protein clean-up solutions

Learn more at thermofisher.com/proteinprep

Thermo Scientific™ Zeba™ Desalting Columns

- Single-use spin columns & filter spin plates
- Re-usable chromatography cartridges
- 7K & 40K molecular weight cut-offs
- Proprietary resin results in excellent protein recovery
- Efficient salt retention (removal) >95%

Thermo Scientific™ Pierce™ Slide-a-Lyzer™ Dialysis Cassettes, MINI devices, and 96-well Microdialysis Plates

- Low-binding plastic and membrane
- Protein recovery >90%
- 2, 3.5, 10 & 20K MWCO
- Sample sizes: 10µl to 250mL
- Secure, validated – no leaks or lost samples

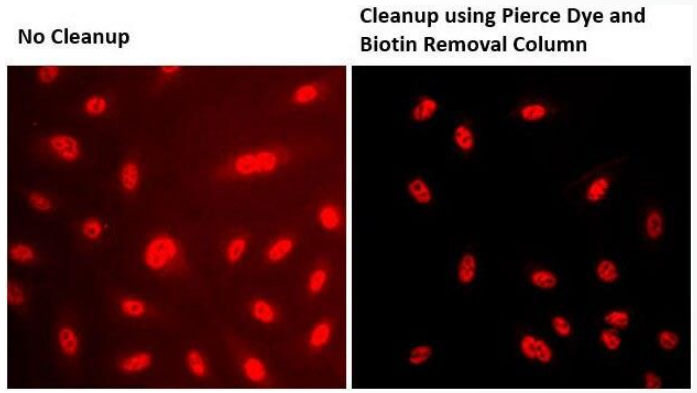
Thermo Scientific™ Pierce™ Concentrators

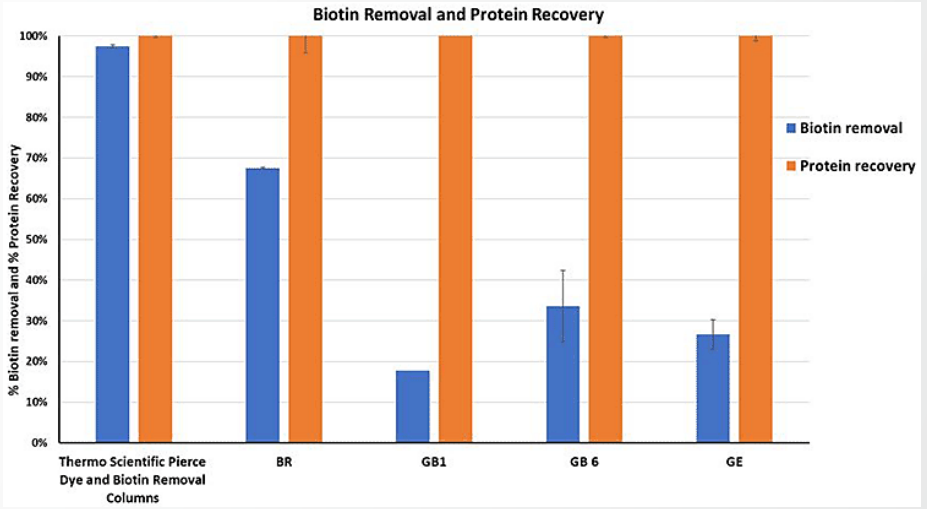
- Concentrate up to 10- to 30-fold in 5-30 min
- Protein recovery >90%
- MWCO's: 3, 5, 10, 30, 50 and 100K
- 0.5, 6, 20, and 100mL sizes
- Use in standard fixed-angle or swinging-bucket centrifuge rotors
- Polyethersulfone (PES) membrane

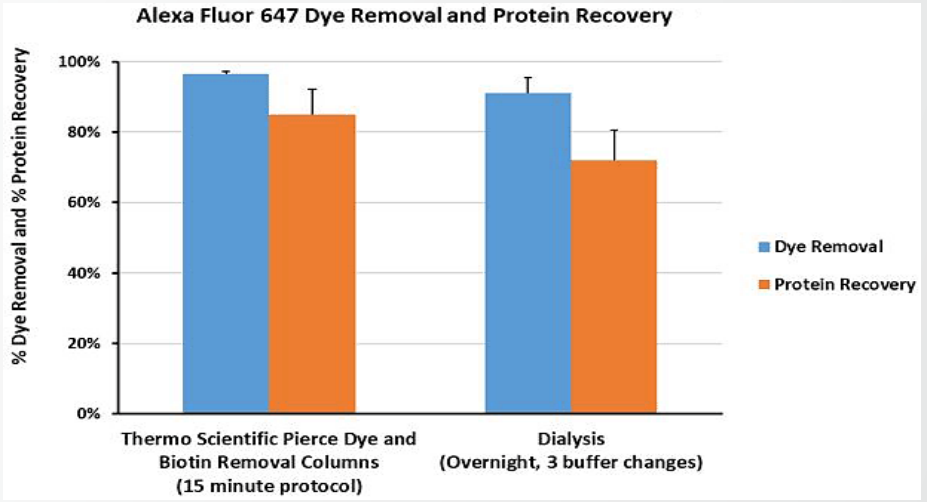
Protein clean-up

Thermo Scientific™ Pierce™ Dye and Biotin Removal Spin Columns and 96-Well Filter Plates

- Removes unreacted fluorescent dyes, biotinylation reagents, crosslinkers & reducing agents from proteins
- Low-binding resin maximizes protein recovery
- No column prep or equilibration required
- Fast – less than 15 minutes
- 0.5, 2, 5, & 10mL spin columns
- Sample sizes range from 50µl to 4mL







Acknowledgments

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  - Wanhua Yan
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  - Kyle Williston

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  - Alexei Yeliseev
  - Klaus Gawrisch

NIH National Institute on Alcohol Abuse and Alcoholism

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Thank you

The line has been unmuted for questions.