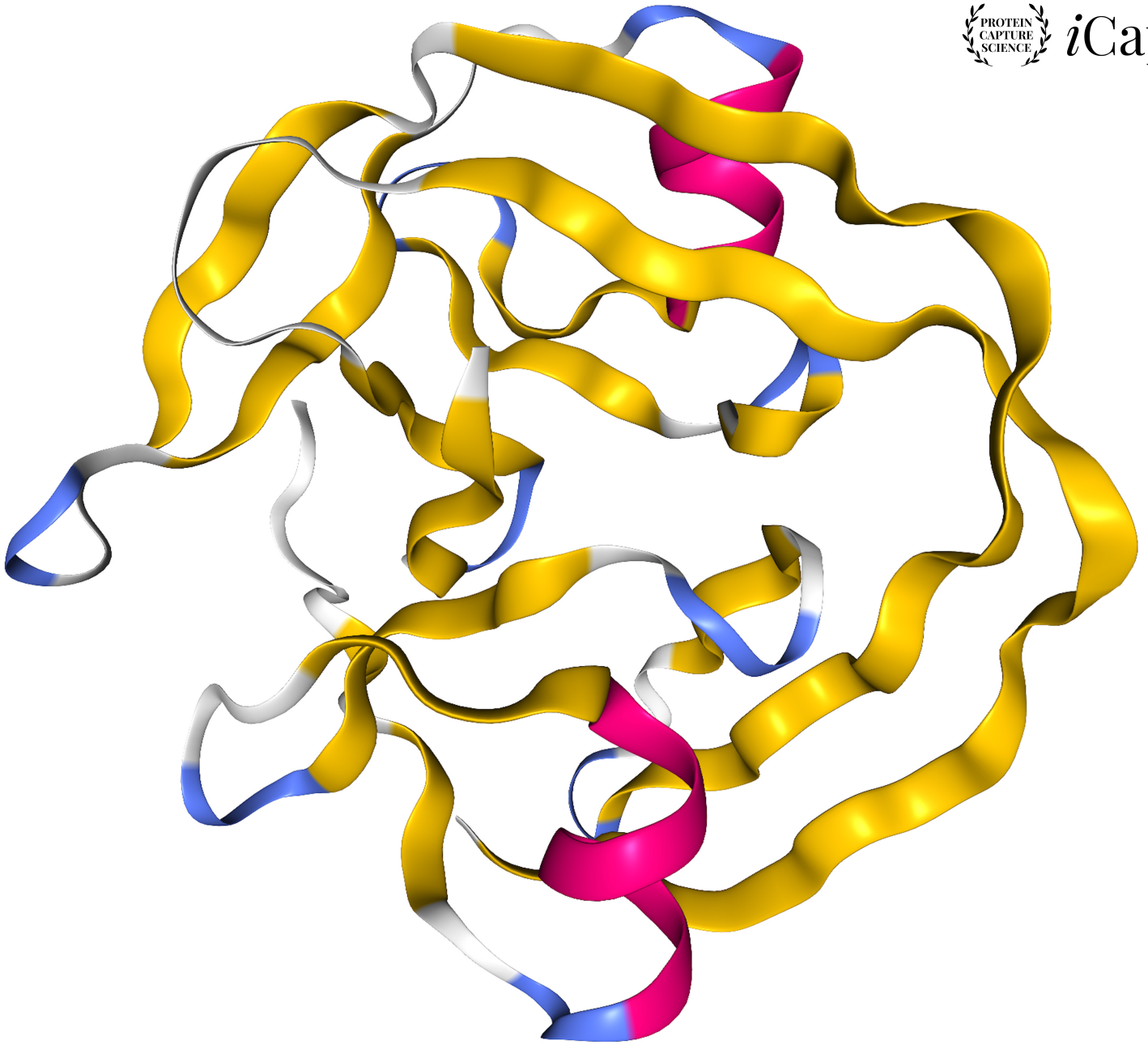


PROTEIN CAPTURE SCIENCE

*i*CapTag™

IMAGINE // DESIGN // INNOVATE // DISCOVER

PROTEIN CAPTURE SCIENCE

*i*CapTag™

A New Platform for the Purification of Tagless Target Proteins

Key *i*CapTag™ Features:

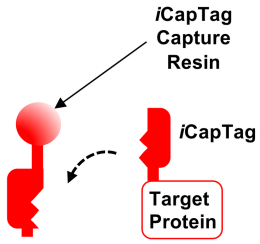
- Single-column purification of tagless target proteins
- Elution of target by on-column tag self-cleavage at mild pH
- No residual amino acids on target
- Cleavage in <5 hours at room temperature
- High purity and yield
- Tunable cleaving performance with minimal modification of target
- Simple resin regeneration protocol
- Compatible with common expression hosts and buffers

PROTEIN CAPTURE SCIENCE

How it works

1

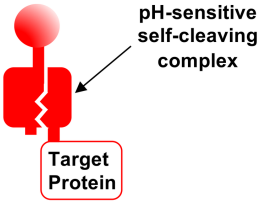
BIND TARGET PROTEIN



The *i*CapTag™ capture resin consists of a simple crosslinked agarose backbone with a covalently bound *i*CapTag™ ligand. The end user appends the 36 amino acid *i*CapTag™ to their protein of interest at its N-terminus, which can then be expressed in the host cell of choice. The clarified feed containing the tagged protein of interest is passed over the *i*CapTag™ resin, where the tag and ligand bind strongly and specifically to each other.

2

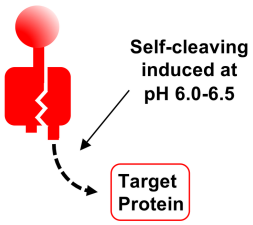
pH 8.5 WASH



Once bound to the *i*CapTag™ resin, the tag and ligand fold together to form a pH-sensitive self-cleaving complex. This allows washing and purification of the bound protein of interest using any of a variety of buffers with a pH of 8.5 or higher. Notably, the stable complex is not affected by salt concentration or other additives, thus allowing significant latitude in developing wash strategies.

3

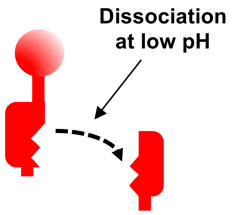
SHIFT pH WAIT ELUTE



Self-cleaving of the tag is induced by a shift in buffer pH from 8.5 to about 6.2, which greatly accelerates the cleaving reaction while leaving the cleaved tag behind on the resin. Cleavage can be carried out in batch mode, where the flow is stopped while the cleavage reaction takes place, or in flow mode, where the cleaved protein of interest is collected as a concentrated peak at the column effluent. The result is a tagless protein recovered in the column effluent.

4

STRIP & REUSE RESIN



Once the purified protein of interest has been collected, the tag can be stripped from the resin by a low-pH wash, typically 150 mM phosphoric acid or similar buffer. The resin is highly reusable, and is stable for caustic wash and sanitization as well. Once stripped, the resin can be reequilibrated in binding buffer and reused.

Construction of the tagged protein of interest

The *i*CapTag™ consists of a modified C-terminal segment of the Npu DnaE intein, where cleavage is mediated by a cyclization reaction of the C-terminal asparagine to form a succinimide ring.

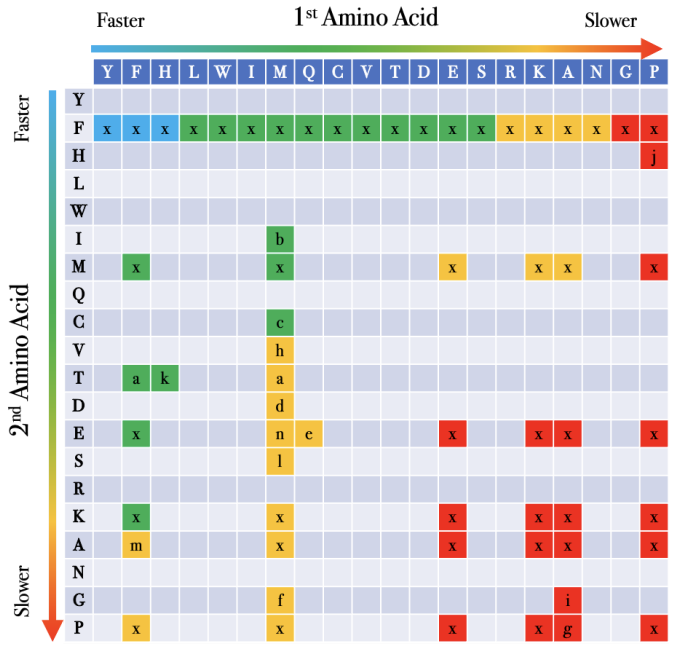
NpuC(HN) tag sequence:
MIKIATRKYLGKQNVYGIGVERDHNFALKNGFIAHN

*i*CapTag amino acid sequence — Protein of interest sequence

MIKIATRKYLGKQNVYGIGVERDHNFALKNGFIAHN $X^{+1}X^{+2}X^{+3}$XXXXXXXXXXXXXX...

Cleavage site

When designing the tagged fusion protein, it is important that the *i*CapTag™ amino acid sequence be joined directly to the first amino acid (X^{+1}) of the protein of interest, with no additional amino acids added. The first two amino acids of the protein of interest have a significant impact on the cleaving rate, where aromatic residues tend to accelerate cleaving while smaller residues slow or stop cleaving and proline abolishes cleaving. For proteins of interest that have unacceptably slow cleaving, amino acids can be added or modified at the N-terminus to provide more optimal cleaving. A full guideline on which amino acids have which effects are included in **Table 1**.



Protein Purified (first three amino acids)			
x	eGFP (modified +1/+2/N)	k	RCNA (H/T/I)
a	G-CSF (modified +1/T/P)	l	TrxA (M/S/D)
b	Streptokinase (M/I/A)	m	NPRT (F/A/S)
c	Mature IFN (M/C/D)	n	DNAJ (M/E/L)
d	Pro-tPA (M/D/A)		
e	Mature tPA (Q/E/I)		
f	Pro-EPO (M/G/V)		
g	Mature EPO (A/P/P)		
h	Pre-pro-fVII (M/V/S)		
i	Pro-fVII (A/G/G)		
j	Mature fVII (P/H/R)		

Table 1. Guidance on addition of first and if needed also second amino acid(s) (X^{+1}/ X^{+2}) to improve cleaving rate effects of designated target proteins based on initial examples of purified proteins. Shaded boxes indicate experimentally verified cleaving rates for the indicated proteins.

Examples of protein purification

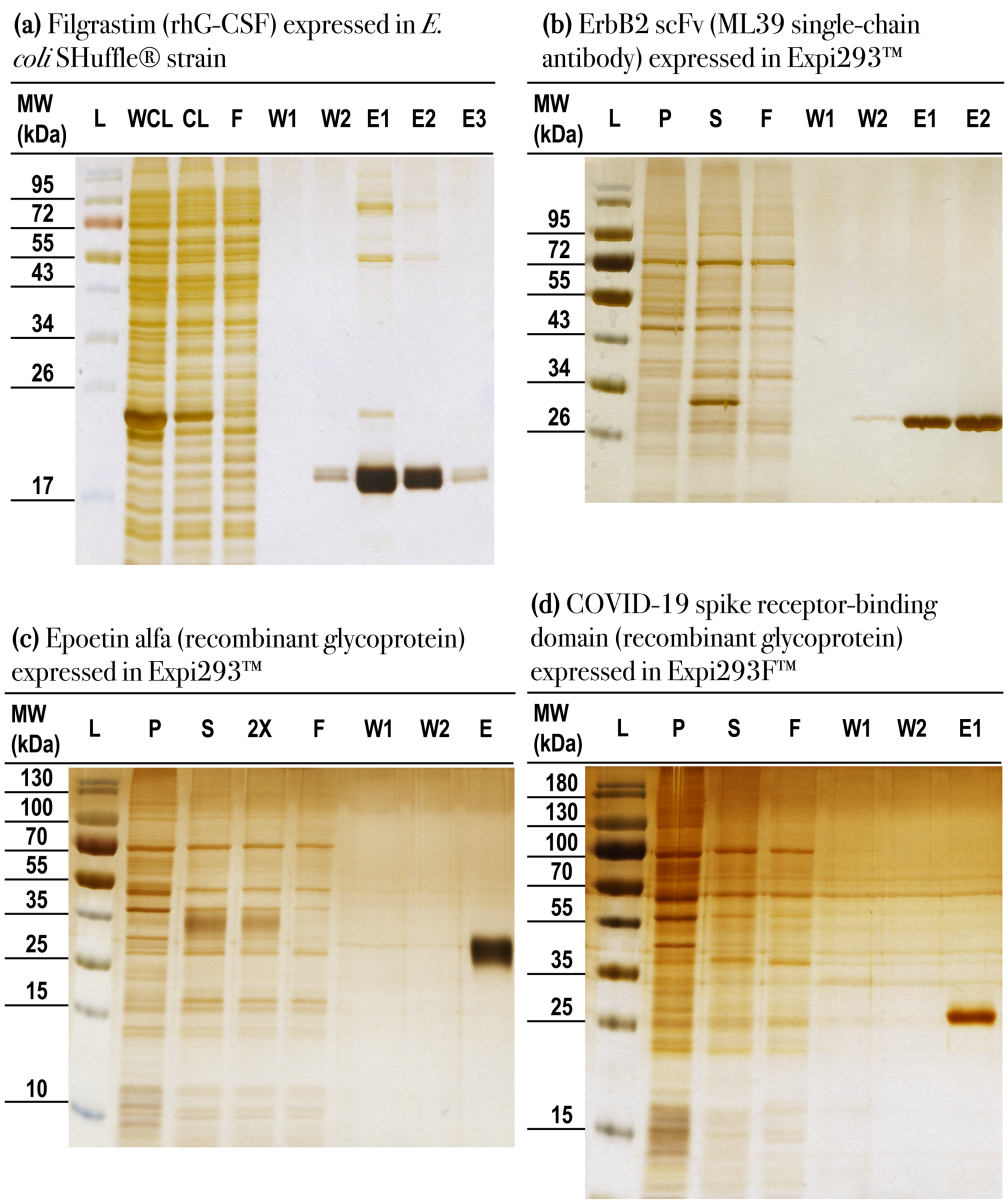


Figure 1. Examples of protein purification using *i*CapTag™ technology; mass spectrometry (MS) analysis indicated glycosylation of erythropoietin (EPO) and Covid spike protein (Courtesy of Dr. Jackelyn M. Galiardi). (a) Filgrastim (rhG-CSF) expressed in E. coli SHuffle® strain; (b) ErbB2 scFv (ML39 single-chain antibody) expressed in Expi293™; (c) Epoetin alfa (recombinant glycoprotein) expressed in Expi293™; (d) COVID-19 spike receptor-binding domain (recombinant glycoprotein) expressed in Expi293F™.

Abbreviations & Notes: L - molecular weight marker, WCL - whole-cell lysate, CL - clarified lysate, F - flow-through, W1 - pH 8.5 wash, W2 - pH 6.2 wash, E - elution fractions, P - cell pellet, S - cell culture supernatant, 2× - 2 × dilution of S, 1–3 - sample numbers, MW - molecular weight. Expi293™ is a trademark of Thermo Fisher Scientific.

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FOR MORE INFORMATION & OPPORTUNITIES TO COLLABORATE CONTACT:

Dr. David W. Wood
CSO, Co-founder

855-PCS-TAGS
(855-727-8247)

dwood@ProteinCaptureScience.com
www.ProteinCaptureScience.com

PROTEIN CAPTURE SCIENCE

PRODUCT PRICING

	Description	Catalog No	No of Items	Price
	**1 mL *i*CapTag™ Prepacked Column** *Free Samples - limited number of items (100 samples)	02000102	1 x 1 mL 1 x 1 mL 5 x 1 mL	$0.00* $245.00 $985.00
	**5 mL *i*CapTag™ Prepacked Column (16 x 26 mm)**	02000502	1 x 5 mL 5 x 5 mL	$895.00 $3,895.00
	**10 mL *i*CapTag™ Bulk Resin**	05001002	1 x 10 mL	$1,890.00
	**25 mL *i*CapTag™ Bulk Resin**	05002502	1 x 25 mL	$4,475.00
	**5 mL *i*CapTag™ Prepacked Column (8 x 100 mm)**	02000502R	1 x 5 mL 3 x 5 mL	$1175.00 $2,895.00
	**The *i*CapTag™ Troubleshooting Kit** 3x1 mL *i*CapTag™ Prepacked Column 1.5 mL *i*CapTag™ Bulk Resin	TKPCS	 3 x 1 mL 1.5 mL	$808.00

THANK YOU FOR YOUR BUSINESS