AFFINITY RESINS

Cube Biotech

PureCube Rho1D4 Agarose

Featuring highly specific binding, the rho1D4 antibody and its epitope are an alternative affinity tag system increasingly used to purify membrane proteins. Using BioWorks Workbeads as the foundation matrix, **Cube Biotech** produces the first commercially available immunoaffinity resin for the rho1D4 purification system.

- demonstrably suitable for membrane protein research
- binding capacity of 3-4 mg protein per mL resin
- purifies protein with high specificity and yield
- gentle protein elution based on competitive binding
- can be regenerated for reuse

The rho1D4 system

An affinity tag system for gentle and highly specific purification of membrane proteins

Rho1D4 refers to the last 9 amino acids of the intracellular C-terminus of bovine rhodopsin. The name comes from the monoclonal antibody that specifically binds to the sequence.[1] Combined with the rho1D4 antibody, this epitope can serve as a highly specific purification tag suitable for membrane proteins.

A membrane protein of interest is genetically modified to incorporate the rho1D4 tag at the C- or N-terminus (Fig. 1). Once outfitted with this sequence, the target protein is captured on an affinity matrix loaded with rho1D4 antibody and subsequently eluted with an excess of rho1D4 peptide. The rho1D4 peptide competitively binds with the matrix antibody, providing for gentler elution conditions than, for example, changing pH (Fig. 2).

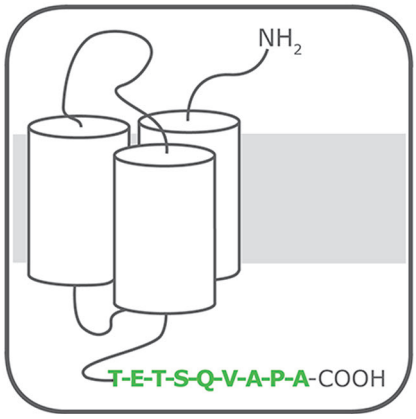


Fig. 1: A hypothetical membrane protein with 3 transmembrane domains. The rho1D4 tag, with sequence T-E-T-S-Q-V-A-P-A, has been added to the C terminus.

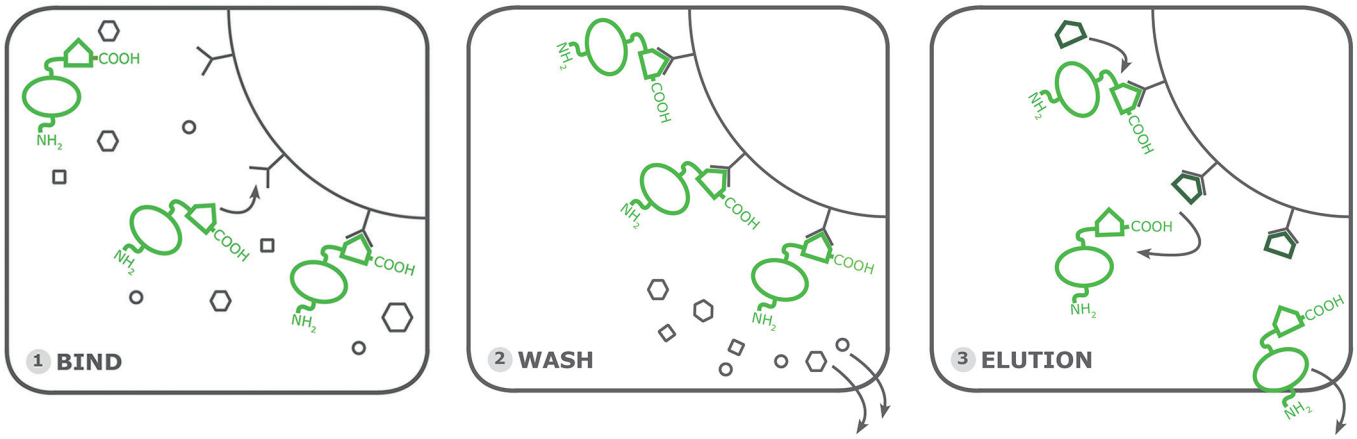


Fig. 2: Like with other affinity tag systems, protein purification with PureCube Rho1D4 is done in a bind-wash-elute procedure. Bind: incubating the rho1D4-tagged protein on a resin loaded with rho1D4 antibody sequesters the target protein from the lysate. **Wash:** unwanted proteins and other lysate components are washed away, leaving the target protein bound to the affinity matrix. **Elution:** excess rho1D4 peptide competitively binds to the matrix and the released target protein is collected in the eluate.

Applications of the rho1D4 system

The rho1D4 epitope and antibody pair was characterized in the 1980's and used to purify bovine rhodopsin expressed in monkey kidney cells by coupling the antibody to Sepharose® beads.[1,2] Since then, the rho1D4 system (tag, antibody-coupled affinity matrix, eluent peptide) has been used to study a handful of membrane proteins including G-protein coupled receptors (GPCR)[5,7–12], an ATP-binding cassette transporter[4,13], a solute counter-transporter[6], and a tetraspanin membrane protein[3].

One advantage of the system is the **high specificity** of the antibody-epitope interaction. Epitope sequence and chain length are critical for binding. For example, replacing the third alanine with glycine which removes a single methyl group, eliminates binding. Likewise, the full 9-amino acid tag binds tightest to the rho1D4 antibody and removing 2 amino acids prevents binding.[1] As a consequence, unspecific binding of proteins containing sequences similar to the rho1D4 epitope is minimized and the purity of the recovered protein is high (Table 1).

Another advantage is the **high yield** of the eluted target protein. Expression systems, including bacterial, yeast, and mammalian cell lines, have been optimized for a selection of GPCRs and other membrane proteins. Purification of the membrane proteins was done with the rho1D4 system followed by gel filtration or centrifugal concentration to remove the eluent peptide. In all systems authors reported recovery of milligram amounts of protein (Table 1).

Finally, the purified proteins can be used for **functional studies**, such as characterization of ligand binding and protein-protein interaction. For example, tagged ABCA4 was immobilized to a matrix loaded with rho1D4 antibody to characterize its binding affinity for its natural ligand (an adduct of retinal), measure its ATPase activity, and assess the functional role of its C-terminus.[2,13] In another example, the protein CD81 was immobilized in its entirety to plates coated with rho1D4 antibody and shown to exhibit the same binding affinity for Hepatitis C Virus envelope E2 protein as the isolated soluble fragment of the protein.[3] Lastly, vesicles reconstituted with the membrane domain of the anion exchanger AE1 tagged and purified with the rho1D4 system showed the same rate of sulfate efflux as vesicles with erythrocyte-sourced AE1.[6]

Protein	Expression system	Purity	Yield (total protein recovered)	Purification steps taken
hVN1R1[9] G protein-coupled receptor	Inducible HEK293S cell line (mammal)	90%	1 mg/1 g cells	Rho1D4 IAC + SEC
FPR3[8] G protein-coupled receptor	Inducible HEK293S cell line (mammal)	90%	2 mg/6 g cells	Rho1D4 IAC + SEC
TAAR5[7] G protein-coupled receptor	Inducible HEK293S cell line (mammal)	90%	1 mg/9 g cells	Rho1D4 IAC + SEC
OR131-2[5] G protein-coupled receptor	Inducible HEK293S cell line (mammal)	85%	2.9 mg/10 g cells	Rho1D4 IAC + centrifugation
hOR17-4[11,12] G protein-coupled receptor	Inducible HEK293S cell line (mammal)	>90%	7.5 mg/2.5 L culture	Rho1D4 IAC + SEC
hOR17-4[10] G protein-coupled receptor	Cell-free wheat germ extract	70%*	0.3 mg/mL reaction solution*	Rho1D4 IAC + SEC
CD81[3] Tetraspanin membrane protein	Inducible HEK293S cell line (mammal)	>95%	26 µg/3X10^7 cells	Rho1D4 IAC
AE1[6] Solute carrier	*S. cerevisiae* strain BJ5457	93%	2.5 mg/18 L culture	Rho1D4 IAC

* Purity was achieved in one step Rho1D4 IAC from low levels in the reaction solution; yield was measured after eluate fractions were concentrated and applied to SEC column.

Table 1. Examples of purity and yield reported in the literature for membrane proteins purified with the rho1D4 system. Although many of the proteins purified with the rho1D4 system have been G protein-coupled receptors, the system has the flexibility to facilitate characterization of other membrane proteins such as transporters. Referenced articles are listed in the literature cited. IAC: immunoaffinity chromatography; SEC: size-exclusion chromatography.

PureCube Rho1D4 System

High quality system components that meet your research needs

Cube Biotech offers the first commercially available components of the rho1D4 system.

- PureCube Rho1D4 Agarose resin
- Rho1D4 peptide
- Rho1D4 antibody

These components can be purchased individually or as part of starter sets, all in conveniently aliquoted sizes and at exceptional prices. To purify proteins from very dilute samples or to conduct pull down experiments, we recommend our **PureCube Rho1D4 MagBeads**.

Purify proteins with high specificity and yield

PureCube resins are produced under strict quality guidelines and each batch undergoes quality checks to ensure that the loaded matrix has a high protein capacity. Combined with the specificity of the antibody-epitope interaction, a purification protocol optimized for the target protein can generate elution fractions with exceptionally high yields. Figure 3 shows a purification run for chemokine receptor 4 (CXCR4). The tagged protein was expressed in *E. coli*, solubilized with Fos-Choline®-14 and purified on a column containing PureCube Rho1D4 Agarose beads. Using the rho1D4 peptide as eluent, the four elution fractions contained a recovered total protein concentration of 0.8, 1.0, 0.85 and 0.6 mg/mL. SDS-PAGE analysis shows CXCR4 at approximately 65 kDa and 35 kDa. These bands represent dimers and monomers of the 39.7 kDa membrane protein. Separation of monomers and dimers as well as removal of the eluent peptide can be done with size-exclusion chromatography.

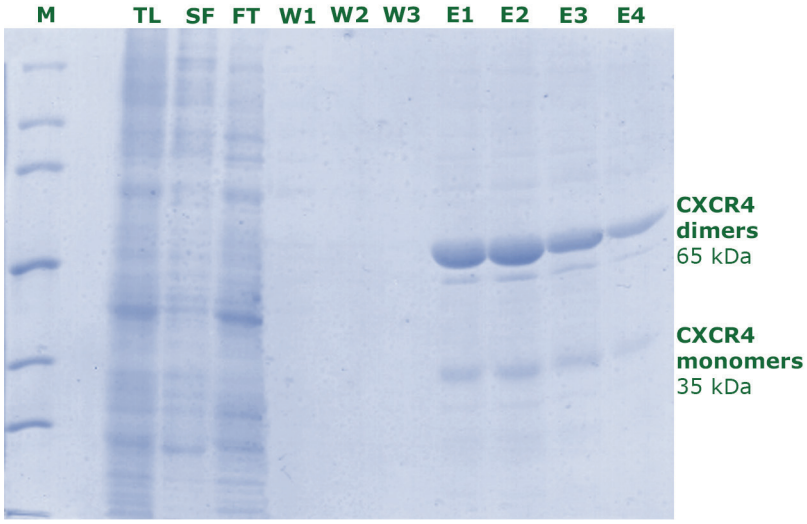
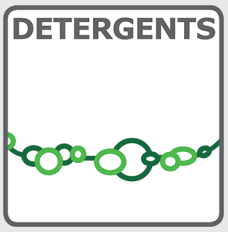


Fig. 3: Purification of chemokine receptor 4 (CXCR4) using PureCube Rho1D4 Agarose. Total lysate (TL) was solubilized with Fos-Choline®-14 and the soluble fraction (SF) was incubated on an immunoaffinity column loaded with rho1D4 antibody. Concentration of eluted CXCR4 ranged 0.6–1.0 mg/mL, as determined spectrophotometrically.

Also available from Cube Biotech:



Our high-quality, ultrapure solubilization agents are an ideal complement to the rho1D4 system.

For sensitive handling and scalable purification of membrane proteins, the rho1D4 system is also available as magnetic beads.

Loaded with a protein-specific ligand, our custom resins enable functional studies and other specialized applications.

Ordering Information

Catalog Number	Product Description
33101	PureCube Rho1D4 Agarose (1 mL) 2 mL 50% suspension
33102	PureCube Rho1D4 Agarose (5 mL) 10 mL 50% suspension
33103	PureCube Rho1D4 Agarose (10 mL) 20 mL 50% suspension
16201	Rho1D4 peptide (5 mg) Sufficient for 1 mL PureCube Rho1D4 Agarose or MagBeads
16203	Rho1D4 peptide (25 mg) Sufficient for 5 mL PureCube Rho1D4 Agarose or MagBeads
33199	Rho1D4 Starter Set 1 PureCube Rho1D4 Agarose (1 mL) and Rho1D4 peptide (5 mg)
40020	Rho1D4 Antibody (200 µg) 50 µL at 4 mg/mL

Also available as magnetic beads!

Literature Cited:

1. Hodges, R.S., et al. 1988. Antigen-antibody interaction. J Biol Chem 263: 11768–11775.
2. Oprian, D.D., et al. 1987. Expression of a synthetic bovine rhodopsin gene in monkey kidney cells. Proc Natl Acad Sci USA 84: 8874–8878.
3. Takayama, H. et al. 2008. High-level expression, single-step immunoaffinity purification and characterization of human tetraspanin membrane protein CD81. PLoS ONE 3: e2314 (DOI: 10.1371/journal.pone.0002314).
4. Zhong, M. and Molday, R.S. 2010. Binding of retinoids to ABCA4, the photoreceptor ABC transporter associated with Stargardt Macular Degeneration. Methods Mol Biol 652: 163–176.
5. Leck, K.-J., et al. 2010. Study of bioengineered zebra fish olfactory receptor 131-2: receptor purification and secondary structure analysis. PLoS ONE 5: e15027 (DOI:10.1371/journal.pone.0015027).
6. Bonar, P. and Casey, J.R. 2010. Purification of functional human Cl-/HCO3- exchanger, AE1, over-expressed in *Saccharomyces cerevisiae*. Protein Express Purif 74: 106–115.
7. Wang, X. et al. 2011. Study of two G-protein coupled receptor variants of human trace amine-associated receptor 5. Sci Rep1: 102 (DOI:10.1038/srep00102).
8. Wang, X. and Zhang, S. 2011. Production of a bioengineered G-protein coupled receptor of human formyl peptide receptor 3. PLoS ONE 6: e23076 (DOI: 10.1371/journal.pone.0023076).
9. Corin, K. et al. 2011. Structure and function analyses of the purified GPCR human vomeronasal type 1 receptor 1. Sci Rep 1: 172 (DOI: 10.1038/srep00172).
10. Kaiser, L., et al. 2008. Efficient cell-free production of olfactory receptors: detergent optimization, structure, and ligand binding analysis. Proc Natl Acad Sci USA 105: 15726–15731.
11. Cook, B. L., et al. 2008. Study of a synthetic human olfactory receptor 17-4: expression and purification from an inducible mammalian cell line. PLoS ONE 3: e2920 (DOI: 10.1371/journal.pone.0002920).
12. Cook, B. L., et al. 2009. Large-scale production and study of a synthetic G protein-coupled receptor: human olfacory receptor 17-4. Proc Natl Acad Sci USA 106: 11925–11930.
13. Zhong, M., et al. 2009. Role of the C terminus of the photoreceptor ABCA4 transporter in protein folding, function, and retinal degenerative diseases. J Biol Chem 284: 3640–3649.

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