RPAS Purification Module

Data File

Affinity Media

The RPAS Purification Module is an integral part of Amersham Biosciences' Recombinant Phage Antibody System (RPAS). The Purification Module is an affinity chromatography kit designed for efficient purification of mouse recombinant Single chain antibody Fragment variable (ScFv) produced in *E. coli.*

- Convenient and fast purification of mouse ScFv using pre-packed affinity column
- No complicated equipment required, syringe or pump operated
- Simple and proven method gives reproducible results
- Pre-made high quality buffer concentrates ensure optimal purification

Fig. 1. RPAS Purification Module.

Introduction

The RPAS Purification Module contains all of the reagents necessary to perform a complete one step purification without using complicated equipment. It also eliminates time consuming processes such as precipitation, dialysis of the sample, buffer preparation and column packing. The Purification Module contains a 5 ml HiTrap column pre-packed with Anti-E Tag Sepharose High Performance media and pre-made Binding, Elution and Neutralizing Buffers for the purification of mouse ScFv antibodies produced, using the pCANTAB 5 E vector.

Using the pCANTAB 5 E vector, soluble functional ScFv-fragments bearing a 13 amino acid peptide tag (E-Tag) are expressed in the *E. coli* periplasm. The soluble ScFv's bearing the E-tag, bind to the Anti-E tag column at neutral pH. Elution is performed by lowering the pH.

See Figure 2 for an overview of the antibody structure. Details of the content of the Purification Module are given in Table 1.

The Purification Module contains buffer for 10 purifications using syringe operation. The optimized matrix, in combination with the design of the column, provide fast preparative purifications with reproducible results within 20–30 minutes.

Table 1. Purification Module

Column:	HiTrap Anti-E Tag, 5 ml	
Binding Buffer	2 × 50 ml, 10 × concentrate containing 20% ethanol as preservative	
Elution Buffer	40 ml, 10 × concentrate	
Neutralizing Buffer	25 ml, containing 20% ethanol as preservative	
Connectors		
Luerlock female/M6 male		1
Luerlock female/M6 female		1
tubing connector flangeless/M6 male		1
tubing connector flangeless/M6 female		1
syringe, domed nut, instructions		1

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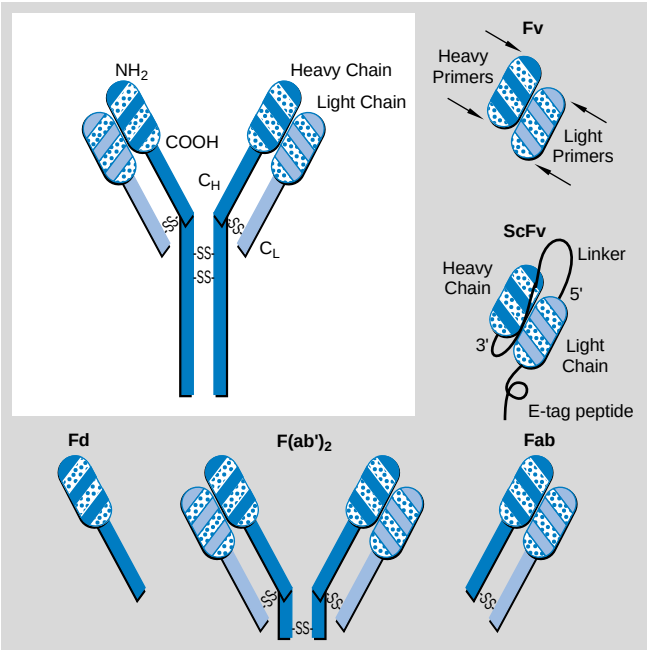


Fig. 2. Antibody model showing subunit composition. Fragments generated by proteolytic cleavage and/or recombinant technology appear in the shaded area.

Matrix

Anti-E Tag Sepharose High Performance is produced by coupling a mouse monoclonal antibody, specific for the 13 amino acid E-Tag, to cross-linked agarose beads by N-hydroxy-succinimide coupling chemistry. The result is a matrix with high performance chromatographic properties.

The mouse antibody has been carefully selected to minimize non-specific cross reactions with *E. coli* proteins. Under the appropriate conditions, the column can be re-cycled in excess of 20 times.

The maximum binding capacity is ≈ 0.7 mg ScFv (MW 30 kDa). The capacity depends however on factors such as the flow rate during sample application and the sample concentration. Detailed characteristics of the HiTrap Anti-E Tag column can be found in Table 2.

Column

The HiTrap column is made of medical grade polypropylene. The column is delivered with a stopper on the inlet and a twist-off end on the outlet. Both ends have M6 connections (6 mm metric threads). The column cannot be opened or refilled. Operation is easy, either using the syringe or, alternatively, with a laboratory pump, e.g. P-1 Peristaltic Pump. A set of connectors is supplied to make it easy to connect the column to different chromatography equipment. These are summarized below.

System/equipment	Connector
Syringe	Luer adaptor female/M6 male
FPLC, GradiFrac systems	Flangeless/M6 male
Low pressure system with capillary tubing	Flangeless/M6 female
Systems with Luer connections	Luer adapter female/M6 male Luer adaptor female/M6 female

Buffers

The Purification Module includes buffers for binding, elution and neutralization. The buffers have been prepared using the highest quality salts and water, and have been filtered through a 0.22 µm filter.

Table 2. HiTrap Anti-E Tag characteristics.

Property	HiTrap Anti-E Tag
Column dimension, d × h	16 × 25 mm
Bed volume	5 ml
Ligand	E-tag specific mouse monoclonal
Binding capacity	≈ 0.7 mg ScFv/column, 20 nmol
Mean bead size	34 µm
Bead structure	Highly cross-linked spherical agarose
Maximum back pressure*	0.3 MPa, 3 bar, 44 psi
Maximum flow rate	20 ml/min
Recommended flow rate	1–5 ml/min
pH stability:	
Short term	2.8–10.5
Storage buffer	12 mM phosphate, 140 mM chloride 0.15% Kathon**, pH 7.4

* Maximum pressure without damage to the column packing.
** Kathon is a trademark owned by Rohm & Haas Inc.

Operation

Complete, easy-to-follow instructions, including sample preparation, purification protocols and trouble shooting guide are included with the Purification Module.

Just a few short steps will give you purified ScFv antibody fragments.

Purification summary:
1. Dilute buffers and prepare *E. coli* periplasmic extract containing soluble ScFv.
2. Equilibrate the column with Binding Buffer.
3. Apply the sample.
4. Wash with Binding Buffer.
5. Elute bound ScFv with Elution Buffer.
6. Re-equilibrate the column with Binding Buffer.

Fig. 3. Syringe operation.

Function testing

The HiTrap anti-E Tag column is tested by affinity chromatography and binds at least 20 nmol of E-Tag peptide/column.

Storage

The entire Purification Module should be stored at +4–8 ˚C.

Applications

The Purification Module is designed for the purification of soluble functional ScFv antibodies, expressed in *E. coli* with the pCANTAB 5 E vector, included in the Expression Module (see Ordering Information). The application shown below illustrates the use of the Purification Module for the isolation of ScFv from a periplasmic extract (Fig. 4). The isolated material was further analysed by gel filtration on Superdex 75, SDS polyacrylamide gel electrophoresis and Western blotting (Figs. 5, 6 and 7).

An example of immunological activity in material purified with the Purification Module is shown in Table 3. ELISA activity was determined in the start, flow through and eluted materials for different sample application volumes.

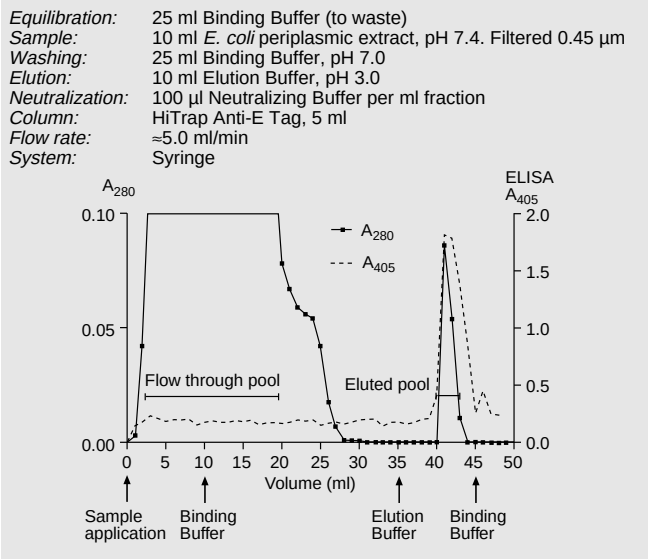


Fig. 4. Purification of anti-lysozyme ScFv from a periplasmic extract with syringe operation.

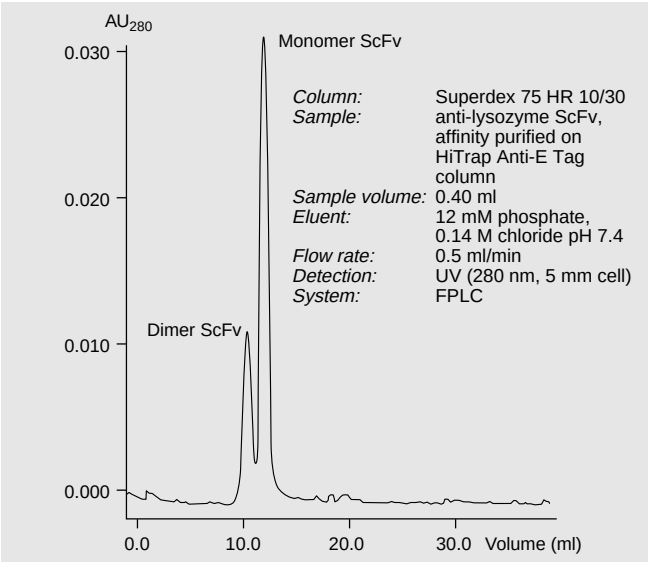
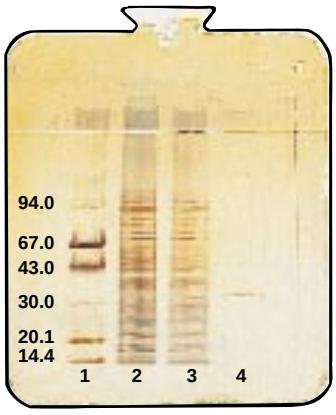


Fig. 5. Size exclusion chromatography on Superdex 75 HR 10/30 of pooled material from HiTrap Anti-E Tag purification of anti-lysozyme ScFv (Fig. 4). Chromatogram shows separation of dimer ScFv from monomeric ScFv.

Table 3. ELISA activity units (activity × dilution × volume) in sample, flow through and in purified material for a ScFv (anti-lysozyme ScFv) purified with the RPAS Purification Module. Microtiter plates were coated with the antigen and the plates were blocked with 3% non-fat dry milk in phosphate buffer saline pH 7.4. Dilution curves of the sample were made in the 3% blocking buffer. Detection was done with the Anti-E Tag Antibody and a goat anti-mouse IgG alkaline phosphate conjugate using pNPP as substrate.

Sample volume ml	ELISA units loaded	ELISA units flow through	ELISA units eluted	Yield %
10	330	0	248	75
20	660	0	627	95
50	1 650	0	1 850	112
100	3 300	124	3 168	96



Lane 1: Low molecular weight calibration kit: 14.4, 20.1, 30.0, 43.0, 67.0, 94.0 kDa
Lane 2: Starting material, periplasmic extract, dil. 1:3
Lane 3: Flow through, dil. 1:2
Lane 4: Eluted ScFv

Fig. 6. Analysis of isolated material from Fig. 4. SDS electrophoresis on PhastSystem using PhastGel 10–15, silver staining.

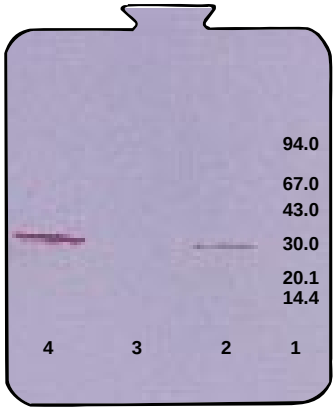


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Fig. 7. Western Blot using PhastTransfer of the above electrophoresis gel (mirror image of Fig. 6). The ScFv was detected with the Anti-E Tag antibody and a goat anti-mouse IgG alkaline phosphate conjugate using BCIP/NBT as substrate.

Trademarks

Ordering information

Product	Code No.	Quantity
Purification Module	17-1362-01	1 kit, 20 purifications
Companion Products		
Mouse ScFv Module	27-9400-01	5 reactions†
Expression Module	27-9401-01	1 kit†
Detection Module	27-9402-01	20 micro titer plates†
Anti-E Tag Antibody	27-9412-01	1 mg†
Anti-E Tag Antibody	27-9412-02	5 mg†
Anti-E Tag Antibody – HRP Conjugate	27-9413-01	0.5 mg
Anti-M 13 Antibody	27-9410-01	1 mg†
Anti-M 13 Antibody	27-9410-02	5 mg†
Anti-M 13 Antibody – HRP Conjugate	27-9411-01	200 micro titer plates†
pCANTAB 5 Gene Rescue Primers	27-1581-01	1 nmol each†
pCANTAB 5 Sequencing Primer Set	27-1585-01	250 pmol each†
Column Accessories		
Domed nut	18-2450-01	4
Union Luerlock		
female/M6 female	18-1027-12	2
female/M6 male	18-1027-62	2
Tubing connector		
flangeless/M6 male	18-1017-98	2
flangeless/M6 female	18-1003-68	2
M6 female/M6 female	19-2143-01	5
Pump P-1, 110/120 V AC	19-4611-02	1
Pump P-1, 210/220 V AC	19-4610-02	1
Superdex 75 HR 10/30	17-1047-01	1

† Product must be shipped cold. There is an extra charge for insulated container and refrigerant.

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