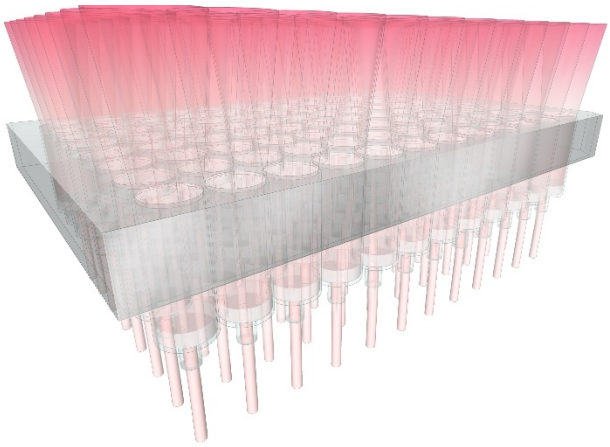
Optima PRO-D Screen
General Instructions for Use

Key Benefits:

- **Instant swelling of resin in a 96 well plate format:** Pre-packed with 50 µl Protein Ark's dried resin formulation.
- **Filtration and purification in one step:** Purify secreted protein from unclarified mammalian or insect cell culture loaded directly into the wells of the screen.
- **Clog-free:** No need for pre-centrifugation or pre-filtration.
- **Drip-free:** The Proprietary SelfSeal Membrane ensures < 2.7% SD for well-well yield reproducibility across the entire plate.

Reproducible contact time: Unique flow control across the entire 96 well plate



The Protein Ark Optima PRO Screen is designed for high throughput protein purification in batch mode. For the first time, you can batch control up to 96 different clarified or unclarified samples with any purification resin, in parallel and with no mess, hands-free and no x-talk between wells.

The Optima PRO Screen Plates are supplied in the following formats:

- Pre-packed with 50 µl super or fastback purification resin and a Diatomaceous Earth filter support (DE) – Optima PRO-D
- Empty
- Pre-packed with 50 µl super or fastback purification resin - Optima PRO

Optima PRO-D screens are pre-packed with 50 µl super or fastback purification resin and a Diatomaceous Earth filter support (DE).
DE is a benign, highly pure filter aid which acts as a permeable filter cake preventing the Optima PRO-D Screen from clogging when unclarified mammalian (HEK/CHO) or insect cells (Sf9/Hi5) are loaded directly into the pre-packed resin wells.

The DE filter support does not affect the purity of the final protein product.

Calibre Scientific UK, Unit 5A, R-Evolution @ The Advanced Manufacturing Park, Selden Way, Rotherham, S60 5XA
Email: info@proteinark.com Telephone: +44 (0) 114 224 2257
Web: www.proteinark.com

	Optima Pro Empty Plate	Optima PRO-D Ni Advance plate	Optima PRO-D Protein A plate	Optima PRO-D Protein G plate	Optima PRO-D Q IEX plate	Optima PRO-D S IEX plate
Resin:	Resin dependent	Fastback Ni Advance	Fastback Protein A	Fastback Protein G	Fastback Q-IEX	Fastback-S-IEX
Specificity:	**Resin dependent**	His-tag	IgG, Fc region	IgG, Fc region	Negatively charged proteins	Positively charged proteins
Matrix:	**Resin dependent**	6% cross-linked Agarose	Sepharose	Sepharose	6% cross-linked agarose	6% cross-linked agarose
Coupled ligand:	**Resin dependent**	Ni chelating ligand	3.5 mg Protein A /ml	2 mg Protein G /ml	Quaternary ammonium	Sulphonic acid propyl
Binding capacity:	**Resin dependent**	80 mg/ml	30 mg/ml	20 mg/ml	50 – 70 mg/ml	50 – 70 mg/ml
Bead size:	**Resin dependent**	90 µm	60 – 165 µm	45 – 165 µm	90 – 100 µm	90 – 100 µm
DTT stability (24 hours)	**Resin dependent**	20 mM	-	-	-	-
EDTA stability (24 hours)	**Resin dependent**	20 mM	-	-	-	-
Buffer compatibility:	**Common aqueous buffers from pH 2-12**	Common aqueous buffers from pH 4-9	Common aqueous buffers from pH 2.5-10	Common aqueous buffers from pH 2-9	Common aqueous buffers from pH 2-12	Common aqueous buffers from pH 2-12
Resin volume per well	**50 µl – 100 µl**	50 µl				
Diatomaceous Earth	No	**Yes**				
Max recommended sample volume	1 ml					
Plastic material	Polypropylene					
Membrane material	PTFE					
Recommended vacuum pressure	-0.6 bar (max. vacuum -0.8 bar)					
Recommended centrifuge speed	2,000 – 3,000 x g					
Recommended shaker speed (2 mm orbit)	800 rpm					
Maximum shaker speed (2 mm orbit)	1,200 rpm					
Shipping/delivery:	Ambient					
Storage temperature:	**Ambient**	2 – 8°C				

Protein Ark

Sample Preparation

Sample Type	Optima PRO-D (pre-packed with resin and Diatomaceous Earth)
Intact Mammalian cells (HEK/CHO)	Unclarified sample (Up to 15 x 10^6 cells/ml)
Intact Insect cells (Sf9/Hi5)	Unclarified sample (Up to 15 x 10^6 cells/ml)
Bacterial cell lysate (*E.coli*)	Pre-clarification of sample (5,000 x g for 15 mins in 2ml deep well block)
Mammalian cell lysate (HEK/CHO)	Pre-clarification of sample (5,000 x g for 15 mins in 2ml deep well block)
Insect cell lysate (Sf9/Hi5)	Pre-clarification of sample (5,000 x g for 15 mins in 2ml deep well block)

1 Packing the Optima PRO empty plate (PAL-HT-EMP-1)

a. Re-suspend the resin (e.g. Super Ni-NTA Resin) by mixing thoroughly to achieve a homogeneous suspension.
b. Add 12 ml 50% suspension into an appropriate container.
c. Add 48 ml of appropriate buffer to obtain a final volume of 60 ml (10% suspension). Gently agitate to achieve a 10% v/v homogeneous suspension.

Note: Proper agitation is necessary to make sure the agarose beads are evenly mixed. If the suspension is not mixed well, agarose beads settle to the bottom of the tube and lead to inconsistent filling and poor well-to-well reproducibility! It is also possible to use 50% suspension, but extra care is required to ensure even distribution of resin in each well.

- Keep mixing the agarose suspension until all wells of the plate are filled

The suspension is considered homogeneous if it appears uniform to the eye. No agarose clumps should be visible

d. Dispense 500 µl suspension into each well of the plate (500 µl 10 % suspension corresponds to 50 µl bed volume).

Note: If you do not use all wells of the plate for purification, seal the top of the empty wells.

e. Centrifuge the Optima PRO plate for 2 mins. at 2,000 x g to remove storage buffer

2 Equilibration

a. Equilibrate resin by adding 500 µl lysis/equilibration buffer to each well.
b. Centrifuge the Optima PRO plate for 2 mins. at 2,000 x g to remove storage buffer

The Optima PRO empty plate is ready for sample loading (step 2 in centrifugation and vacuum protocol)

Protein Ark

Optima PRO-D
Purification using 5-step 25 min Centrifugation Protocol

1. Setting Up
    a. Tap the Optima PRO-D screen plate to ensure all resin/DE pellets are at the bottom of each well.
    b. Gently remove the ClearVue seal from the bottom and top of the Optima PRO-D screen plate and place on to the shaker frame.

2. Sample Loading
    a. Load up to 1 ml of clarified or unclarified sample in each well and incubate for 15 mins, shaking at 800 rpm (2mm orbit shaker).

3. Sample Flowthrough
    a. Transfer the Optima PRO-D screen plate on to a 2 ml wash plate and centrifuge for 2 mins. at 2,000 x g.

4. Washing
    a. Add up to 1 ml of binding/wash buffer to each well and centrifuge at 2,000 x g for 2 min. Repeat the wash step twice.

5. Elution:
    a. Transfer the Optima PRO-D screen plate on to a 0.35 ml collection plate. Add up to 300 µl elution buffer to each well and centrifuge at 2,000 x g for 2 min. Repeat the elution step, if necessary.
    b. Store the eluted protein at 2-8 °C until further analyses.

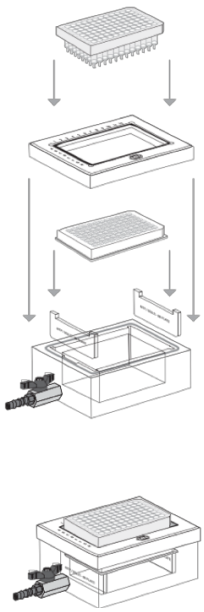
Notes
- Ensure full mixing of the sample, resin and DE is achieved during the incubation step. Shaking speed can be increased up to 1200 rpm (shaker orbit 2 mm).
- Increase incubation time to increase resident time between sample and resin for improved binding and purification.
- Number of wash and elution steps should be optimized for each protein purification.
- The recommended minimum elution volume is 50 µl.

Protein Ark

Purification Protocol using Vacuum Manifold

Setting up the vacuum manifold (Fig. 1)

Equilibration/Washing Steps



Step 4: Place the Optima PRO Screen plate on top of the manifold lid

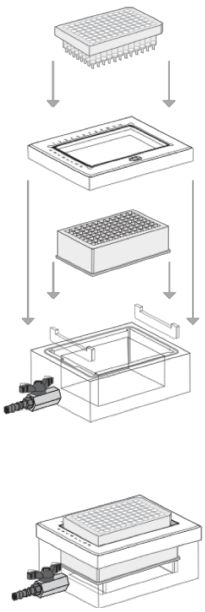
Step 3: Place the manifold lid on top of the manifold base

Step 2: Place the vacuum wash plate in the manifold

Step 1: Insert spacers in the manifold base

Final setup

Elution Step



Step 4: Place the Optima PRO Screen plate on top of the manifold lid

Step 3: Place the manifold lid on top of the manifold base

Step 2: Place the square-well block in the manifold

Step 1: Insert square-well block spacers in the manifold base

Final setup

1 Setting up the vacuum manifold for the preparation of the Optima PRO-D screen plate

a. Insert spacers into the grooves located on the short ends of the manifold.
b. Insert the waste container into manifold base.
c. Place the pressure sealing plate/shaker base on to the spacers inside the manifold base. Close the manifold base with the manifold lid.

2. Sample loading

a. Place the Optima PRO-D screen plate into the shaker frame.
b. Load up to 1 ml clarified or unclarified sample in each well and incubate for 15 mins., shaking at 800 rpm (2mm orbit shaker).

3. Sample Flow-Through

a. Place the Optima PRO-D screen plate on top of the manifold base as illustrated in Fig. 1.
b. Apply approx. -0.6 bar vacuum until all wells have drained. If necessary, press down the plate slightly until flow through starts. *Note: The vacuum may have to be adjusted for optimal results.*
c. Apply approx. -0.8 bar vacuum for a few seconds to remove any residual drips from nozzles. When all the sample has flow through the plate, release the vacuum.

4. Washing

a. Wash the resin by adding up to 1 ml wash buffer to each well.
b. Apply approx. -0.6 bar vacuum for 1 min.
c. Allow the buffer to pass through the wells.

d. Apply approx. -0.8 bar vacuum of for a few seconds to remove any residual drips from the long drip nozzles. Release the vacuum. Repeat the washing step twice. Remove the Optima PRO screen plate from the vacuum manifold.

5. Set up the Vacuum Manifold for Elution
   a. Remove manifold lid, wash plate and waste container from the vacuum manifold.
   b. Insert new spacers for square-well blocks into the grooves located at the narrow ends of the manifold.
   c. Insert a square-well block into the base of the manifold.
   d. Add the lid to the manifold base.

6. Elution
   a. Add up to 300 µl elution buffer to each well.
   b. Place the plate on to the top of the manifold base.
   c. Elute the target proteins by applying approx. -0.6 bar vacuum for 1 min.
   d. Apply approx. -0.8 bar vacuum for a few seconds to remove any drops from the long drip nozzles.
   e. Release the vacuum. Repeat the elution step, if necessary. Store the eluted protein at 2-8 °C until further analyses.

Notes

- Ensure full mixing of the sample, resin and DE is achieved during the incubation step. Shaking speed can be increased up to 1200 rpm (shaker orbit 2 mm).
- Increase incubation time to increase resident time between sample and resin for improved binding and purification.
- Number of wash and elution steps should be optimized for each protein purification.
- The recommended minimum elution volume is 50 µl.

Ordering Information

Product Description	Product Codes		
	1 kit	2 kits	10 kits
Optima PRO Empty 96 well Screen	PAL-HT-EMP-1	PAL-HT-EMP-2	PAL-HT-EMP-10
Optima PRO-**D** Ni Advance 96 well Screen	PAL-HT-NiADV-1D	PAL-HT-NiADV-2D	PAL-HT-NiADV-10D
Optima PRO-**D** Ni NTA 96 well Screen	PAL-HT-NiNTA-1D	PAL-HT-NiNTA-2D	PAL-HT-NiNTA-10D
Optima PRO-**D** Protein A 96 well Screen	PAL-HT-PA-1D	PAL-HT-PA-2D	PAL-HT-PA-10D
Optima PRO-**D** Protein G 96 well Screen	PAL-HT-PG-1D	PAL-HT-PG-2D	PAL-HT-PG-10D
Optima PRO-**D** Q-IEX 96 well Screen	PAL-HT-Q-1D	PAL-HT-Q-2D	PAL-HT-Q-10D
Optima PRO-**D** S-IEX 96 well Screen	PAL-HT-S-1D	PAL-HT-S-2D	PAL-HT-S-10D

Kit components/compositions

Component	Pack Sizes		
Optima PRO Screen plate	1 pc	2 pc	10 pc
Pressure sealing/vacuum plate	1 pc	2 pc	2 pc
2 ml wash collection plate	2 pc	4 pc	-
0.35 ml eluate collection plate	1 pc	2 pc	-
Hanging drop blotting membrane	2 pc	4 pc	20 pc
Sealing mats	1 pc	2 pc	10 pc

2019 Vs 2.2

Calibre Scientific UK, Unit 5A, R-Evolution @ The Advanced Manufacturing Park, Selden Way, Rotherham, S60 5XA

Email: info@proteinark.com Telephone: +44 (0) 114 224 2257
Web: www.proteinark.com