PureSpeed™ Tips

For Protein Purification

Introduction

Rainin PureSpeed Protein Tips are available with three types of resin: Pro A, Pro G and Ni-IMAC

Each type of tip is available in two formats, "200" with a maximum sample volume of 200 µL, and "1000" with a maximum volume of 1000 µL. For each 200 PureSpeed tip, the resin bed volume is 5 or 20 µL, bound by a retention mesh that minimizes dead space For the 1000 PureSpeed tips, the resing bed is 20 or 80 µL. Integrated design of the PureSpeed tip and resin bed insure maximum capture potential and protein elution for the affinity resin. The maximum efficiency of capture and elution of the specific protein(s) of interest can be achieved using the specified protocol – see next page.

Storage

Store the tips at 4° C after receipt; all other PureSpeed components can be stored at room temperature.

METTLER TOLEDO

PureSpeed Protein Tips are packaged with glycerol to protect the resin from drying. After shipping, small droplets of glycerol may be seen on the tip walls as well as in the clamshell. See Figure 1 below. These droplets are normal and will not affect a tip's performance.

Figure 1
Typical clamshell after shipping showing droplets of glycerol inside clamshell and tips

Buffer Preparation.

Note: Below, sodium phosphate buffer (pH 7.4) refers to a solution containing Na_2HPO4 and NaH_2PO4 mixed at the appropriate proportion to maintain the solution pH at 7.4. Sodium phosphate buffer (pH 2.5) refers to a solution mixture of NaH_2PO4 and H_3PO4 in the proportion that brings the solution pH to 2.5. Tris buffer (pH 9.0) and Tris buffer (pH 7.4) refer to solutions of Tris base and Tris-HCl mixed in proportion to bring the solution pH to 9.0 and 7.4, respectively. See www.mt.com/purespeed for a link to a guide to making buffers.

Recommended Buffer for ProA / ProG resins

5X Equilibration/Capture/Wash 1 buffer:

50 mM sodium dihydrogen phosphate buffer (pH 7.4), 700 mM NaCl

1X Equilibration buffer:
10 mM sodium dihydrogen phosphate buffer (pH 7.4), 140 mM NaCl

1X Capture buffer:
10 mM sodium dihydrogen phosphate buffer (pH 7.4), 140 mM NaCl

1X Wash 1 buffer:
10 mM sodium dihydrogen phosphate buffer (pH 7.4), 140 mM NaCl

1X Wash 2 buffer:
140 mM NaCl

1X Elution buffer:
200 mM sodium dihydrogen phosphate buffer (pH 2.5), 140 mM NaCl

1X Neutralization buffer:
1 M Tris buffer (pH 9.0)

Notes:

All buffers listed for PureSpeed ProA/ProG tips are at pH 7.4 as this allows for optimal binding of antibodies to the ProA or ProG resin. The 5X Equilibration/Capture/Wash 1 buffer is included to make the preparation of 1X Equilibration, Capture, and Wash 1 buffers more convenient. Regarding equilibration, PureSpeed tips are shipped in glycerol and may be used without prior treatment; however, treatment with Equilibration buffer is recommended for samples sensitive to glycerol. For Wash 2, the buffer used in this step is unbuffered and used to remove the first wash solution from the resin. This step makes elution by the low pH Elution buffer easier and more reproducible. After purification of IgG with PureSpeed ProA or ProG resins, use Neutralization buffer to normalize the solution pH. After elution, add Neutralization buffer at 25 % v/v of Elution buffer volume (eg: 5 µL Neutralization buffer for a 20 µL antibody sample in Elution buffer).

Recommended Buffer for IMAC resin

5X Equilibration/Capture/Wash 1 buffer:

50 mM sodium phosphate buffer (pH 7.4), 1.5 M NaCl, 25 mM imidazole

5X Wash 2 buffer:
50 mM sodium phosphate buffer (pH 7.4), 1.5 M NaCl, 100 mM imidazole

1X Equilibration buffer:
10 mM sodium phosphate buffer (pH 7.4), 300 mM NaCl, 5 mM imidazole

1X Capture buffer:
10 mM sodium phosphate buffer (7.4), 300 mM NaCl, 5 mM imidazole

1X Wash 1 buffer:
10 mM sodium phosphate buffer (pH 7.4), 300 mM NaCl, 5 mM imidazole

1X Wash 2 buffer:
10 mM sodium phosphate buffer (pH 7.4), 300 mM NaCl, 20 mM imidazole

1X Elution buffer:
10 mM sodium phosphate buffer (pH 7.4), 140 mM NaCl, 250 mM imidazole

Notes:

All buffers listed for PureSpeed IMAC tips are at pH 7.4 as this allows for optimal binding of 6xHis protein to the IMAC resin. The 5X Equilibration/Capture/Wash 1 and 5X Wash 2 buffers are listed above to make preparation of 1X Equilibration, Capture, Wash 1, and Wash 2 buffers more convenient. Regarding equilibration, PureSpeed tips are shipped in glycerol and may be used without prior treatment; however, treatment with Equilibration buffer is recommended for samples sensitive to glycerol. As for PureSpeed tip washing steps, it is possible that the NaCl and imidazole concentrations need to be optimized to obtain the desired target protein purity. Care should be taken, because as NaCl and imidazole concentrations increase, the retention of the target protein on the resin and final protein yield might decrease. For the elution step, up to 500 mM imidazole and NaCl can be used in the Elution buffer for higher protein yield; however, doing this might lead to lower protein purity. Finally, in the case that the initial sample contains calcium salts, substitute Tris buffer (pH 7.4) for sodium phosphate buffer (pH 7.4) in Equilibration, Capture, and Wash 1 buffers. Calcium forms insoluble hydroxyapatite precipitates in the presence of phosphate and this occurrence may preclude successful protein purification.

Charts showing various standardized protocol buffer volumes

Pro A				
Tip Volume	200 µL		1000 µL	
Resin µL	5	20	20	80
Equilibration µL	100	100	500	500
Capture sample µL*	10-200	10-200	100-1000	100-1000
Wash I µL	100	100	500	500
Wash II µL	100	100	500	500
Elution µL	15	60	60	240

Pro G				
Tip Volume	200 µL		1000 µL	
Resin µL	5	20	20	80
Equilibration µL	100	100	500	500
Capture sample µL*	10-200	10-200	100-1000	100-1000
Wash I µL	100	100	500	500
Wash II µL	100	100	500	500
Elution µL	15	60	60	240

Ni-IMAC				
Tip Volume	200 µL		1000 µL	
Resin µL	5	20	20	80
Equilibration µL	100	100	500	500
Capture sample µL*	10-200	10-200	100-1000	100-1000
Wash I µL	100	100	500	500
Wash II µL	100	100	500	500
Elution µL	15	60	60	240

*For larger samples use multiple aliquots in the Capture step (e.g. Capture 2x, Capture 3x, etc.)

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