SpinOUT™: Buffer Exchange

G-Biosciences

Abstract

SpinOUT™ protocol for buffer exchange of peptide and protein samples.

(Cat. # 786-170 to 786-173, 786-703 to 786-708, 786-865 to 786-869)

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Guidelines

INTRODUCTION

The SpinOUT™ columns are versatile, spin-format columns for the desalting and buffer exchange of peptide and protein and other macromolecule solutions ranging from 5µl through to 3ml sample volumes. The SpinOUT™ columns are available in three MWCO sizes for >700, >6,000 or >30,000 dalton peptides or proteins and are suitable for samples containing as little as 20µg peptide or protein/ml. The

SpinOUT™ columns are simple to use as the peptide or protein solution is applied and then centrifuged to recover protein with the column retaining >95% of the salts and small molecules (<100Da for SpinOUT™ GT-100, <1,000Da for Spinout™ GT-600 and <1,500 for Spinout™ GT-1200).

ITEMS SUPPLIED

Cat. #	Description	# Supplied	Resin Bed Volume (ml)	Sample Load Volume (ml)
786-865	SpinOUT™ GT-100, 0.1ml	25	0.1	0.005-0.03
786-866	SpinOUT™ GT-100, 1ml	10	1	0.03-0.3
786-867	SpinOUT™ GT-100, 3ml	10	3	0.3-0.9
786-868	SpinOUT™ GT-100, 5ml	5	5	0.75-1.5
786-869	SpinOUT™ GT-100, 10ml	5	10	1-3
786-703	SpinOUT™ GT-600, 0.1ml	25	0.1	0.005-0.03
786-170	SpinOUT™ GT-600, 1ml	10	1	0.03-0.3
786-171	SpinOUT™ GT-600, 3ml	10	3	0.3-0.9

786-704	SpinOUT™ GT-600, 5ml	5	5	0.75-1.5
786-705	SpinOUT™ GT-600, 10ml	5	10	1-3
786-706	SpinOUT™ GT-1200, 0.1ml	25	0.1	0.005-0.03
786-172	SpinOUT™ GT-1200, 1ml	10	1	0.03-0.3
786-173	SpinOUT™ GT-1200, 3ml	10	3	0.3-0.9
786-707	SpinOUT™ GT-1200, 5ml	5	5	0.75-1.5
786-708	SpinOUT™ GT-1200, 10ml	5	10	1-3

STORAGE CONDITIONS

The columns are shipped at ambient temperature. Upon arrival, store the columns at 4˚C. If stored and handled correctly the columns have a shelf-life of 1 year.

SPECIFICATIONS

SpinOUT™ GT-100

• Particle size: 55-165µm

• Exclusion limit (Mr): 700

SpinOUT™ GT-600

• Particle size: 20-130µm

• Exclusion limit (Mr): 6,000

SpinOUT™ GT-1200

• Particle size: 35-200µm

• Exclusion limit (Mr): 30,000

IMPORTANT INFORMATION

Sample Load Volume

The recommended load volumes (see table) are a guideline. The actual volumes used will be dependent on your sample, the concentration of salts and contaminants to be removed and the recovered purity desired. For optimal removal of contaminants, we recommend using a sample volume of <20% of the resin bed volume.

NOTE: Loading more than the recommended load volume will result in a higher level of contaminating salts and other molecules.

ADDITIONAL ITEMS NEEDED

• Variable speed centrifuge

• 1.5-2ml microcentrifuge collection tubes for the 0.1ml (Cat. # 786-703, 786-706) and 1ml (Cat. # 786-170, 786-172) spin columns

• 15ml collection tubes for the 3ml (Cat. # 786-171, 786-173) and 5ml (Cat. # 786- 704, 786-707) spin columns

• 50ml collection tubes for the 10 ml (Cat. # 786-705, 786-708) spin columns

• Buffer for buffer-exchange

Table 1: Centrifugation times for optimal sample recovery.

Column size	Centrifugation Time (mins)
0.1ml column	4
1ml column	4
3ml column	6
5ml column	8
10ml column	10

Materials

SpinOUT™ Columns 786-170 by G-Biosciences

Protocol

PREPARATION BEFORE USE

Step 1.

Mark one side of the column and ensure in all centrifugations the mark is facing outwards during centrifugation.

PREPARATION BEFORE USE

Step 2.

Prepare the SpinOUT™ column by centrifuging the SpinOUT™ columns at 1,000g for 1 minute to compact the resin.

DURATION

00:01:00

PREPARATION BEFORE USE

Step 3.

Remove the top and then bottom caps. Place into an appropriate collection tube.

PREPARATION BEFORE USE

Step 4.

Centrifuge the column at 1,000g for 2 minutes to remove the storage buffer.

DURATION

00:02:00

Step 5.

Place the column in a new collection tube and remove the cap.

Step 6.

Add the buffer to be exchanged into to the column

a. For 0.1ml column, use 75µl buffer

b. For 1ml column, use 0.5ml buffer

c. For 3ml column, use 1.5ml buffer

d. For 5ml column, use 2.5ml buffer

e. For 10ml column, use 5ml buffer

Step 7.

Centrifuge the column at 1,000g for 2 minutes to remove the buffer.

DURATION

00:02:00

Step 8.

Repeat steps 6 and 7, a further five more times, ensuring the buffer is discarded after each centrifugation.

Step 9.

Place the column in a new collection tube and remove the cap.

Step 10.

Slowly, apply the protein solution to the center of the SpinOUT™ resin. The recommended volumes to apply to the column are as follows:

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NOTES

Colin Heath 15 Jun 2016

Sample Load Volume

The recommended load volumes (see table) are a guideline. The actual volumes used will be dependent on your sample, the concentration of salts and contaminants to beremoved and the recovered purity desired. For optimal removal of contaminants, we recommend using a sample volume of <20% of the resin bed volume.

NOTE: Loading more than the recommended load volume will result in a higher level of contaminating salts and other molecules.

Step 11.

Centrifuge the column at 1,000g for the indicated times in the table below to collect the protein solution. Discard the column.

Column size	Centrifugation Time (mins)
0.1ml column	4
1ml column	4
3ml column	6
5ml column	8
10ml column	10