

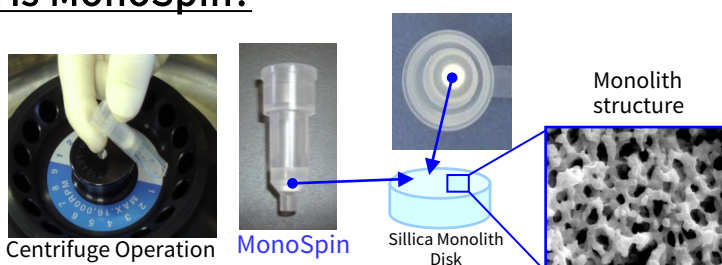
Reversed-phase HPLC is used for the quantitative analysis of proteins. Proteins are separated based on the difference of hydrophobicity. Sample preparation is necessary when analyzing biological samples to reduce the affect from matrix.

Purification of biological samples using revers-phase solid phase extraction requires acetonitrile or methanol contained solvent for analyte elution. However, there is a case high recovery can't be obtained due to a few factors: slow partition, precipitation by high concentration of organic solvent and strong adsorption to packing material.

In this note, MonoSpin, uniquely suited for handling small amount samples, is used to purify the proteins. During the process, changes in selectivity and recovery are seen due to the usage of different kind of organic solvent. (Y. Yui, S.Ota)

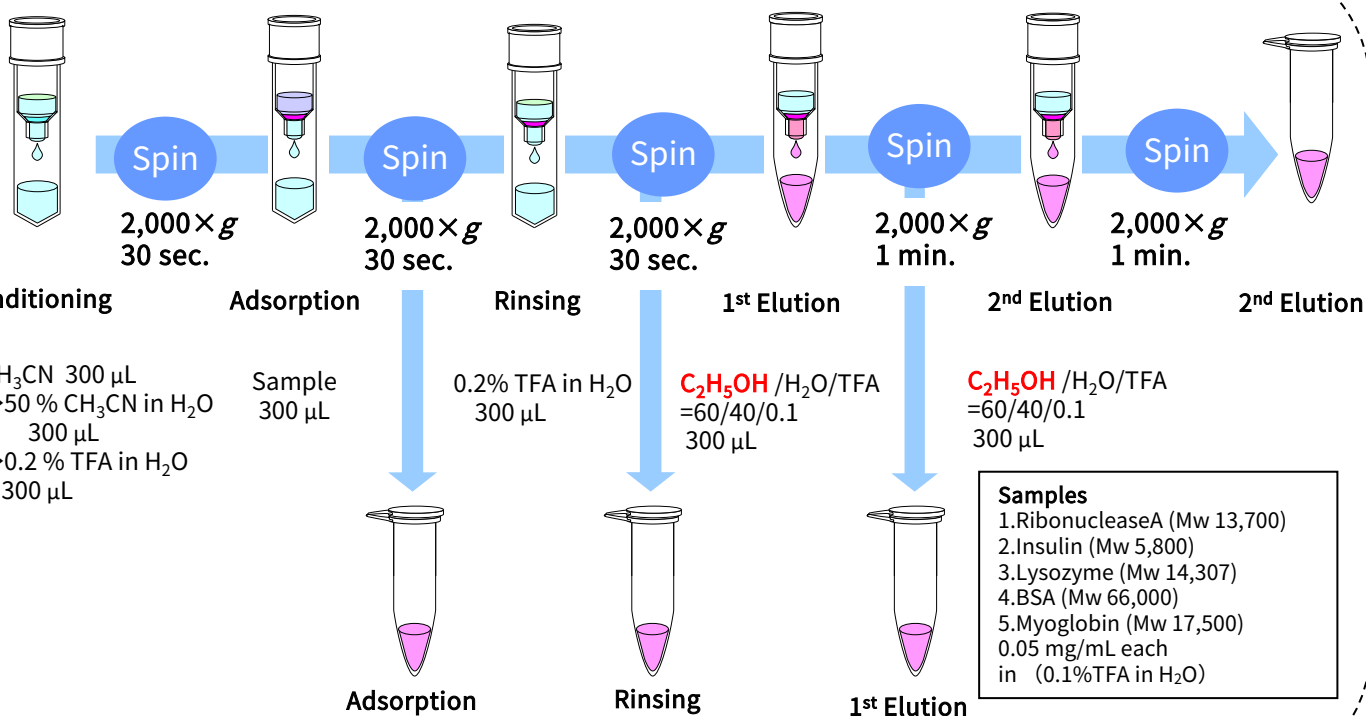
What is MonoSpin?

MonoSpin SPE centrifugal spin columns were developed to improve concentration of small-volume sample preparation. The low-pressure, high-flow, and low-liquid-retention properties of GL Sciences' monolith silica technology makes it uniquely suited for handling small amount samples.



Operation of MonoSpin C18

Ethanol is used for elution

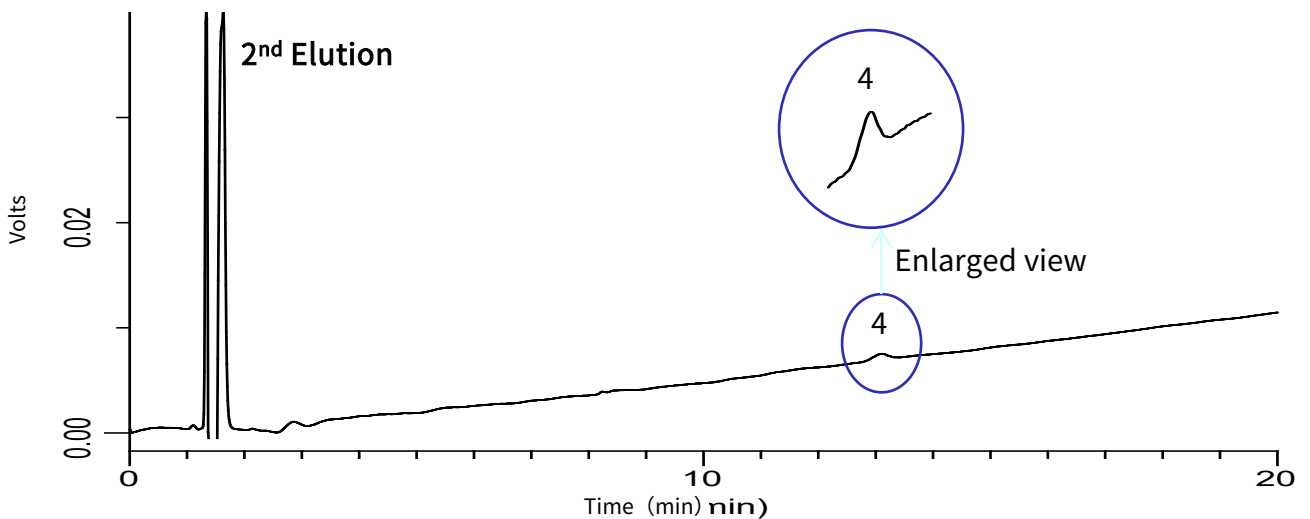
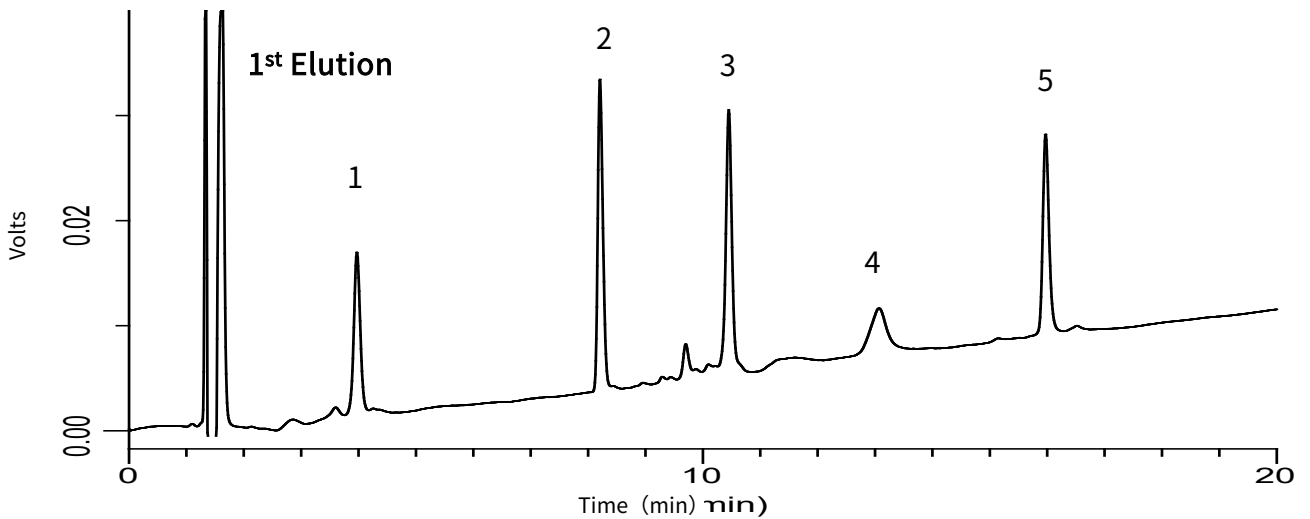
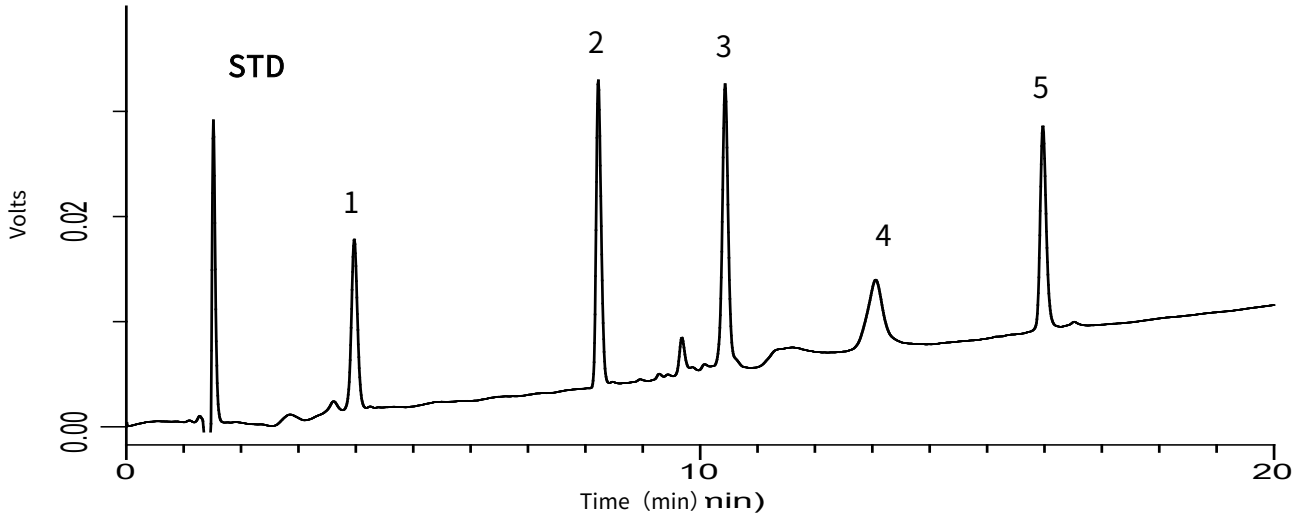


Chromatogram

- 1. RibonucleaseA (Mw 13,700)
- 2. Insulin (MW 5,800)
- 3. Lysozyme (MW 14,307)
- 4. BSA (MW 66,000)
- 5. Myoglobin (MW 17,500)

Conditions

Column: InertSustainBio C18 (1.9 μm, 100 × 2.1 mm I.D.)
 Eluent: A) 0.1 % TFA in H₂O
 B) 0.1 % TFA in CH₃CN
 A/B=72/28 - 20min - 45/55 - 0.1 min - 72/28 - 5 min
 Flow rate: 0.2 mL/min
 Col.Temp: 40 °C
 Detection: UV 220 nm
 Injection Vol: 2 μL



Recovery (%)

Recovery ($n=3$) is calculated by the peak area. Only BSA isn't recovered enough compared to other proteins. However, it makes possible to increase recovery by eluting twice.

Purification Process Compounds	Recovery (%)			
	Adsorption	Rinsing	1 st Elution	2 nd Elution
RibonucleaseA	0	0	101.0	0
Insulin	0	0	104.9	0
Lysozyme	3.6	0	91.8	0
BSA	11.5	0	75.7	7.8
Myoglobin	0	0	99.9	0

Also, poor recovery is seen when eluting with acetonitrile or methanol. Recovery rate changes depending on elution solvent.

Acetonitrile elution

Acetonitrile is used at 1st and 2nd elution instead of ethanol.

Purification Process Compounds	Recovery (%)			
	Adsorption	Rinsing	1 st Elution	2 nd Elution
RibonucleaseA	0	0	12.8	8.4
Insulin	0	0	107.7	2.0
Lysozyme	2.5	0	93.8	8.5
BSA	9.7	0	60.9	6.3
Myoglobin	0	0	97.3	7.8

Methanol elution

Methanol is used at 1st and 2nd elution instead of ethanol

Purification Process Compounds	Recovery (%)			
	Adsorption	Rinsing	1 st Elution	2 nd Elution
RibonucleaseA	0	0	83.2	8.7
Insulin	0	0	94.8	0.9
Lysozyme	4.6	0	0	0
BSA	12.0	0	0	0
Myoglobin	0	0	0	1.3

Products



MonoSpin C18

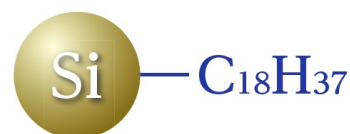
Cat. No. 5010-21700 (50 pcs)
Cat. No. 5010-21701 (100 pcs)

*We have trial kit for initiating your analysis.

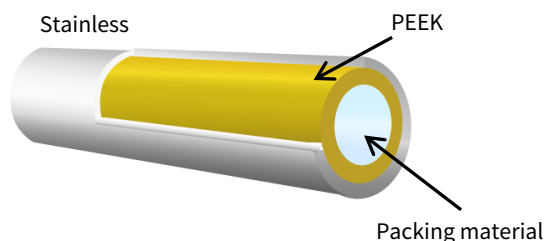
MonoSpin Customize 20:	You may choose 2 kinds of MonoSpin (10 pcs each) Trypsin is an exception Cat. No. 5010-01001
MonoSpin Trial 1 :	Appropriate for pesticide (C18, SCX, SAX, TiO /10 pcs) Cat. No. 5010-21740
MonoSpin Trial 2 :	Appropriate for sugars and hydrophilic compounds. (C18, Amide, CBA, NH ₂ /10 pcs) Cat. No. 5010-21741
MonoSpin Trial 3 :	Appropriate for ionic compounds (SCX, SAX, CBA, NH ₂ /10pcs) Cat. No. 5010-21742

HPLC • LC/MS Column InertSustainBio C18

Rapid Separations of Proteins and Peptides



Column sectional view



Features

- Separation of compounds from small to large molecules
- A radically new Steel-Coated-PEEK hardware preventing adsorption of peaks
- Suitable for analyzing Phosphate compounds

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