# Monolith SPE Column for Sample Preparation

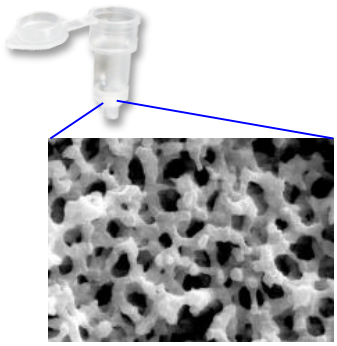
## Monolith SPE Column for the Purification and Enrichment of Trace Amount Sample

## MonoSpin® Series

GL Sciences' silica monolith, created synthetically using ethyl silicate, has a very uniform three dimensional structure that shows excellent reproducibility from batch-to-batch.

The solid structure of GL Sciences' silica monolith eliminates the need for frits or filters at the ends of the column, thereby reducing dead volume that might otherwise lead to band broadening or sample recovery. For example, when used in the form of a spin column, samples loaded in 10 µL volume, rinsed, and eluted with 10 µL elution buffer show excellent recoveries.

MonoSpin Column



Monolith structure

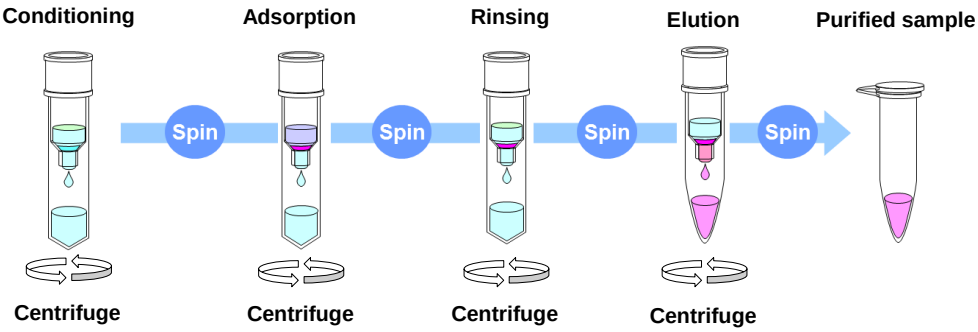
## Features

### Easy to Operate

Centrifuge elution allows loss-free and efficient processing of many samples simultaneously, with little or no liquid retained by the separation matrix.

### Fast

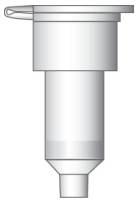
Excellent mass transfer and rapid sample binding MonoSpin's monolith silica allows extremely rapid sample preparation compared with other methods.




Centrifuge Operation

### Ideal for Small Sample Volumes

The small type is excellent for the pretreatment for samples of 50-800 µL and the large type for samples of 500-8000 µL (0.5-8 mL).



**Small type**

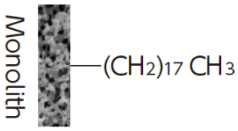
- Column Size : Φ 4.2 x 1.5 mm
- Sample Volume : 50 - 800 µL
- Elution Volume : 50 - 800 µL
- Centrifugation speed : 2,000 - 10,000 x g

**Large type**

- Column Size: Φ 9 x 3 mm
- Sample Volume : 500 - 8000 µL
- Elution Solvent : 500 - 8000 µL
- Centrifugation speed : 1,000 x g

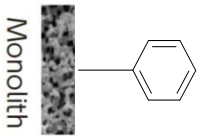
# MonoSpin® Series Product Lineup

## MonoSpin® C18/C18 FF S L *



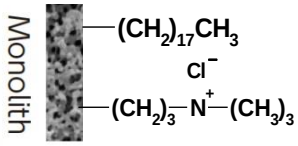

Octadecyl functional group.
Optimal for drug extraction in biological samples, and desalting & enrichment of peptide samples.
C18 FF employs large through-pore monolith silica for high viscosity samples.

## MonoSpin® Ph S



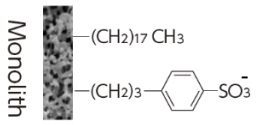

Phenyl functional group.
Optimal for the recovery of hydrophobic drugs in biological samples due to its weak retentivity and different selectivity compared to a C18 phase.

## MonoSpin® C18-AX S



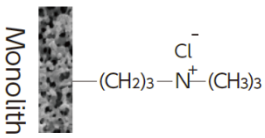

Bonded with octadecyl and trimethylaminopropyl, a mixed mode type. Delivers great retention for high-salt concentrated serum samples. Optimal for the recovery of acidic drugs.

## MonoSpin® C18-CX S



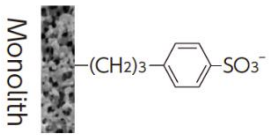

Bonded with octadecyl and benzene sulfonic acid combining both ion exchange & hydrophobic interaction. Optimal for dissociated basic drug in biological samples. Delivers higher cleanup efficiency compared C18 or SCX.

## MonoSpin® SAX S L



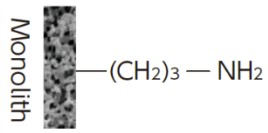

Bonded with trimethyaminopropyl combining both strong anion exchange & weak hydrophobic interaction. Optimal for the extraction of acidic drugs.

## MonoSpin® SCX S L



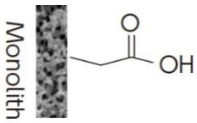

Bonded with benzenesulfonic acid combining both strong cation exchange & hydrophobic interaction. Optimal for the extraction of basic drugs.

## MonoSpin® NH2 S L



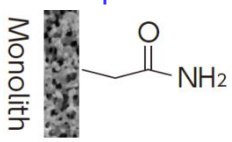

Bonded with aminopropyl.
Optimal for the enrichment of sugar chain and/or hydrophilic compounds by HILIC mode.

## MonoSpin® CBA S L



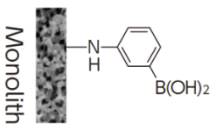

Bonded with carboxyl acid combining both weak cation exchange. Optimal for the extraction of basic drugs.

## MonoSpin® Amide S



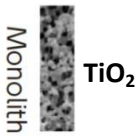

Bonded with amide.
Optimal for the extraction of sugar chains and various hydrophilic acidic and basic compounds by HILIC mode.

## MonoSpin® PBA S



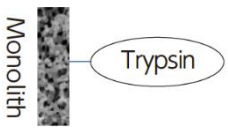

Specific column combined with phenyl boric acid. Excellent for the selective extraction of cis diol compounds, such as catechol amines.

## MonoSpin® TiO S



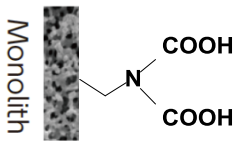

Monolith skeleton coated with titanium dioxide. Excellent for the enrichment of phosphopeptides.

## MonoSpin® Trypsin S



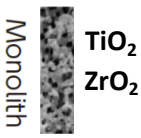

Bonded trypsin is available for performing rapid and efficient tryptic digests of proteins.

## MonoSpin® ME S L .....



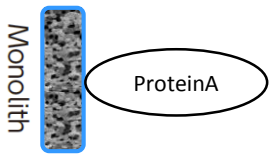

Bonded with iminodiacetic acid.
Optimal for the recovery of trace metals.

## MonoSpin® Phospholipid S L ....



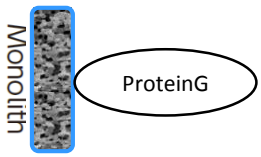

Monolith skeleton coated with $TiO_2$ and $ZrO_2$. Excellent for the adsorption and removal of phosphopeptides.

## MonoSpin® ProA S 96 .....




Protein A immobilized affinity spin column for the rapid purification of antibodies.

## MonoSpin® ProG S 96 .....




Protein G immobilized affinity spin column for the rapid purification of antibodies.

S : Small type  L : Large type  96 : 96-well plate

# Specification of MonoSpin® Series

## Specifications of MonoSpin® Series

| Products | Stationary Phases | Small type | | Large type | | Surface area ($m^2/g$) | Maximum loading volume (Small type) | Filter |
|---|---|---|---|---|---|---|---|---|
| | | Through-pore (μm) | Meso-pore (nm) | Through-pore (μm) | Meso-pore (nm) | | | |
| MonoSpin® C18 | Octadecyl | 5 | 10 | 10 | 10 | 350 | 100 μg（Amitriptyline） | NA |
| MonoSpin® C18 FF | Octadecyl | 20 | 15 | - | - | 300 | 50 μg（Amitriptyline） | |
| MonoSpin® Ph | Phenyl | 5 | 10 | - | - | 350 | 100 μg（Amitriptyline） | |
| MonoSpin® C18-AX | Octadecyl and trimethylaminopropyl | 5 | 10 | - | - | 350 | 100 μg（Ibuprofen） | |
| MonoSpin® C18-CX | Octadecyl and benzenesulfonic acid | 5 | 10 | - | - | 350 | 100 μg（Amitriptyline） | |
| MonoSpin® SAX | Trimethylaminopropyl | 5 | 10 | 10 | 10 | 350 | 100 μg（Ibuprofen） | |
| MonoSpin® SCX | Benzenesulfonic acid | 5 | 10 | 10 | 10 | 350 | 100 μg（Amitriptyline） | |
| MonoSpin® NH2 | Aminopropyl | 5 | 10 | 10 | 10 | 350 | 100 μg（Maltopentaose） | |
| MonoSpin® CBA | Carboxyl acid | 5 | 10 | 10 | 10 | 350 | 100 μg（Amitriptyline） | |
| MonoSpin® Amide | Amide | 5 | 10 | - | - | 350 | 100 μg ( Angiotensin Ⅱ ) | |
| MonoSpin® PBA | Phenyl boric acid | 5 | 10 | - | - | 350 | 100 μg (Dopamine) | |
| MonoSpin® TiO | Titanium dioxide | 20 | 15 | - | - | 350 | 40 μg (Adenosine monophosphate) | |
| MonoSpin® Trypsin | TPCK* treated Trypsin | 5 | 10 | - | - | 350 | - | |
| MonoSpin® ME | Iminodiacetic acid | 5 | 10 | 10 | 10 | 350 | 25 μg (Cu ion) | |
| MonoSpin® Phospholipid | $TiO_2$ and $ZrO_2$ | 5 | 10 | 10 | 10 | 350 | 10 μL (Human serum) | |
| MonoSpin® ProA | Protein A | 2 | 60 | - | - | - | 400 μg (Human IgG) | |
| MonoSpin® ProG | Protein G | 2 | 60 | - | - | - | 400 μg (Human IgG) | |

* TPCK: L-(tosylamido-2-phenyl) ethyl chloromethyl ketone.

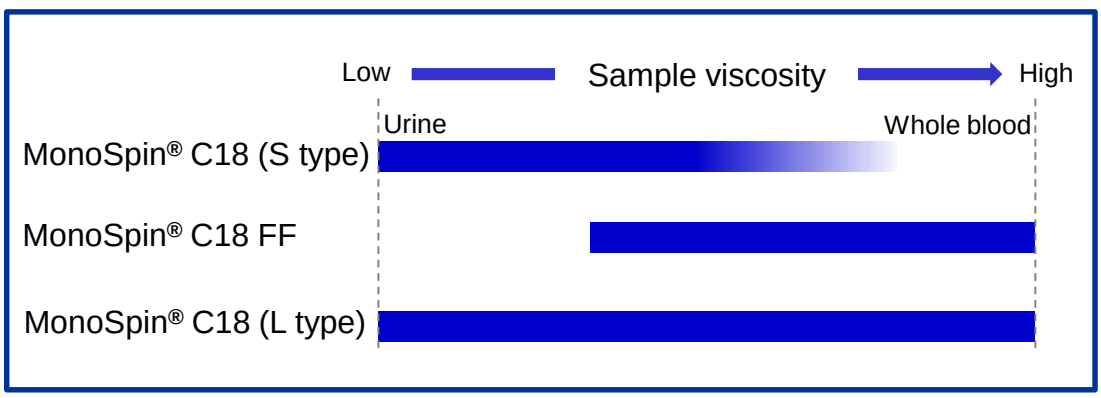
## Specifications of MonoSpin® Column Types

| Description | MonoSpin® Small Type*1 | MonoSpin® Fast Flow (FF)*2 | MonoSpin® Large Type |
|---|---|---|---|
| Column size | Φ 4.2 x 1.5 mm | Φ 4.2 x 1.5 mm | Φ 9 x 3 mm |
| Sample volume | 50 - 800 μL | 50 - 800 μL | 500 - 8000 μL |
| Elution volume | 50 - 800 μL | 50 - 800 μL | 500 - 8000 μL |
| Centrifugation speed | 2,000 - 10,000 ×*g* | 1,000 ×*g* | 1,000 ×*g* |
| Maximum loading volume | 100 μg | 50 μg | 1 mg |

＊1: MonoSpin® ProA and MonoSpin® ProG have different specifications. For more details, please see page xx.
＊2: Fast Flow type (FF) is only available for MonoSpin® C18.

## For Various Viscosity Samples

MonoSpin® series are ideal for the sample preparation of biological samples. MonoSpin® C18 Fast Flow (FF) type is appropriate for high viscosity biological samples. Select the appropriate MonoSpin® column type depending on the viscosity of sample and volume.



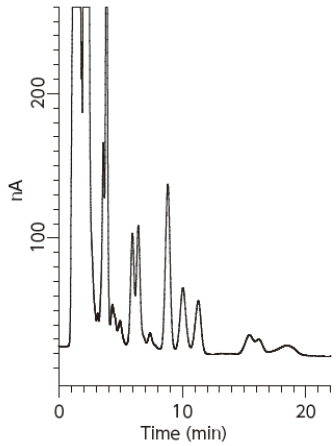
# Application with MonoSpin® Series

## Purification and Enrichment of Trace Amount Sample

The low-pressure, high-flow, and-liquid-retention properties of GL Sciences' monolith silica technology make it uniquely suited for handling of small samples. MonoSpin® SPE centrifugal spin columns have been developed to improve concentration and yields in low-volume sample preparation without requiring evaporation or reconstitution.

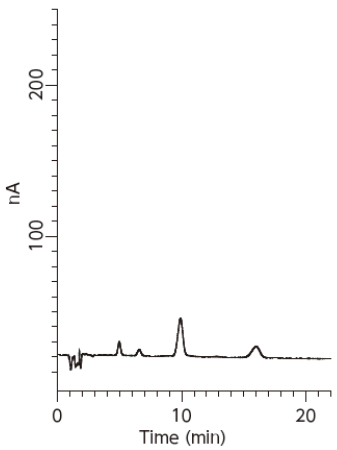
### Small type columns

Without MonoSpin® PBA

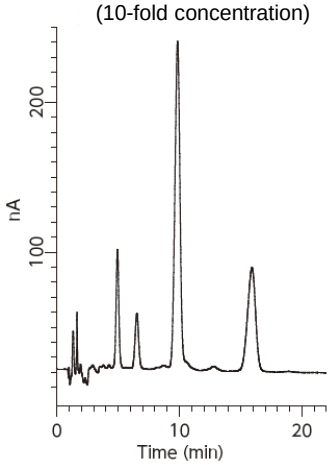



Purification using MonoSpin® PBA
Sample volume : 500 µL

(1) Elution volume : 500 µL




(2) Elution volume : 50 µL
(10-fold concentration)



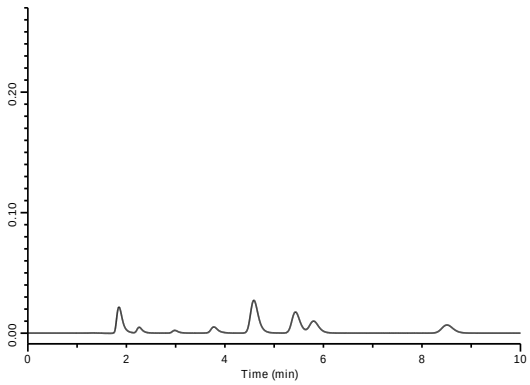

**Conditions**

Column : Inertsil ODS-3 (5 µm, 150 × 2.1 mm I.D.)
Eluent : 50 mM Phosphate buffer (pH 5.6)
50 mg/LEDTA, 600 mg/L IPCC-008, -10% Methanol
Flow Rate : 0.3 mL/min

Col.Temp : 35 ℃
Injection Vol. : 5 µL
Detection : ECD Pulse Mode

### Large type columns

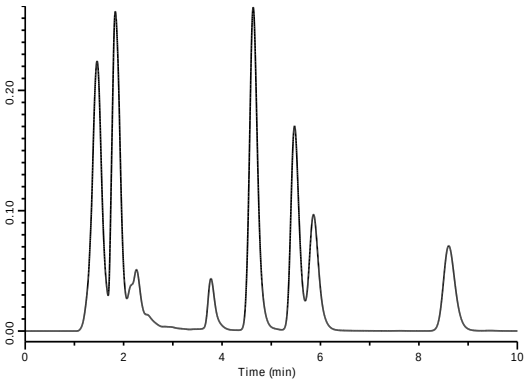
Without MonoSpin® C18




Purification of serum sample using MonoSpin® C18
Sample volume : 500 µL

5000 µL serum sample is purified with MonoSpin® C18 and is enriched by 500 µL .

Elution volume : 500 µL (10-fold concentration)




**Conditions**

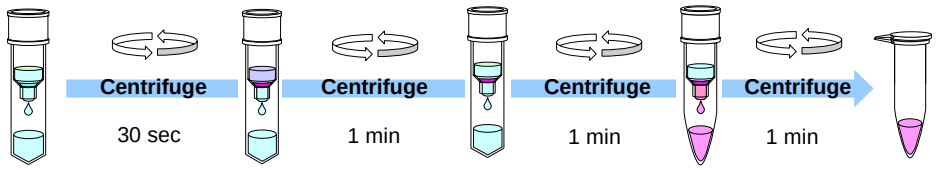
Column : Inertsil ODS-3 (5 µm, 150 × 4.6 mm I.D.)
Eluent : $CH_3CN$/3 mM $KH_2PO_4$, 27 mM SDS = 48/52
Flow Rate : 1.0 mL/min

Col.Temp : 40 ℃
Injection Vol. : 1 µL
Detection : 230 nm

# Application with MonoSpin® C18

## Purification of Amphetamine in Urine Using MonoSpin® C18

**Centrifuge** : 5,000 × *g*




**Sample preparation**

Prepare 800 µL sample solution mixed with 400 µL urine and 400 µL buffer solution (pH 13.0)

**Conditioning**

Add 100 µL methanol
↓ Centrifuge
Add 300 µL buffer solution (pH 13.0)

**Adsorption**

Add 800 µL sample solution

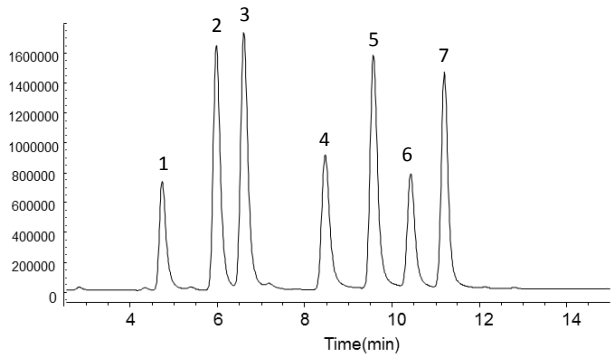
**Rinsing**

Add 300 µL buffer solution (pH 13.0)

**Elution**

Add 100 µL 0.1 % formic acid – methanol ( 1 : 1, v/v ).

**Purified sample**



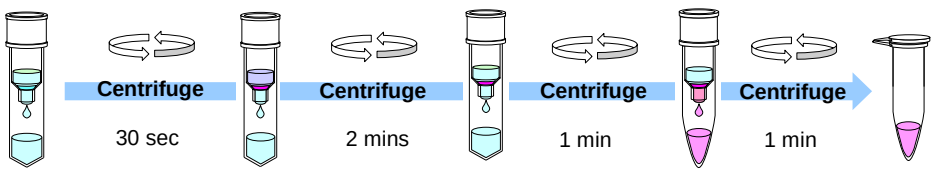

Data provided by Dr. Namera of Hiroshima University.

**Conditions**

Column : InertSustainSwift C18 (3 µm, 150 × 2.1 mm I.D.)
Eluent : A) 10 mM Ammonium acetate – Formic acid (pH 3.3)
B) $CH_3OH$
A/B = 90/10 - 2 min - 90/10 - 13 min - 70/30,v/v
Flow Rate : 0.3 mL/min
Col. Temp. : 40 ℃
Detection : LC/MS
Sample : 1. Norephedrine
2. Ephedrine
3. Methylephedrine
4. Amphetamine
5. Methamphetamine
6. 3,4-methylenedioxyamphetamine
7. 3,4-methylenedioxymethamphetamine

## Recovery of Drugs in Serum using MonoSpin® C18

**Centrifuge** : 2,300 × *g*




**Sample preparation**

Prepare 600 µL sample solution mixed with 200 µL serum and 400 µL 10 mM potassium phosphate buffer solution (pH 7.0)

**Conditioning**

Add 300 µL methanol
↓ Centrifuge
Add 300 µL 10 mM potassium phosphate (pH 7.0)

**Adsorption**

Add 600 µL sample solution

**Rinsing**

Add 300 µL water

**Elution**

Add 200 µL acetonitrile

**Purified sample**

Reproducibility study for drugs in serum sample for three days using MonoSpin® C18 (n=10).
MonoSpin® C18 demonstrated high reproducibility and recovery, but also superb purification efficiency for drugs in serum sample.

**Con. : Concentration**

| Sample | Con. (ng/mL) | Recovery (%) | RSD (%) |
|---|---|---|---|
| Desipramine | 5 | 91.2 | 4.8 |
| | 10 | 86.1 | 3.3 |
| | 50 | 85.2 | 5.9 |
| | 250 | 88.4 | 6.5 |
| Imipramine | 5 | 96.3 | 9.5 |
| | 10 | 95.8 | 1.5 |
| | 50 | 94.5 | 0.9 |
| | 250 | 95.9 | 0.9 |
| Fluvoxamine | 5 | 96.8 | 11.6 |
| | 10 | 87.1 | 5.0 |
| | 50 | 86.8 | 8.1 |
| | 250 | 87.5 | 9.7 |

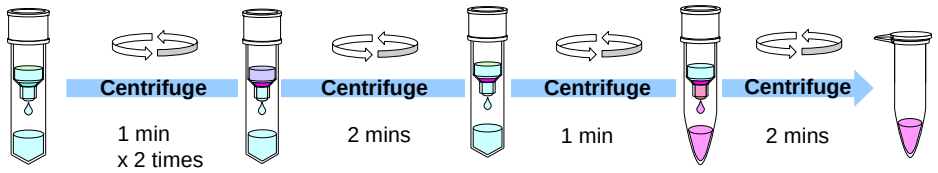
| Sample | Con. (ng/mL) | Recovery (%) | RSD (%) |
|---|---|---|---|
| Paroxetine | 5 | 83.7 | 3.9 |
| | 10 | 84.1 | 7.8 |
| | 50 | 83.9 | 8.2 |
| | 250 | 86.7 | 7.5 |
| Maprotiline | 5 | 85.7 | 8.1 |
| | 10 | 84.7 | 3.2 |
| | 50 | 88.6 | 5.4 |
| | 250 | 87.5 | 7.7 |
| Duloxetine | 5 | 106.3 | 9.9 |
| | 10 | 104.8 | 6.7 |
| | 50 | 99.8 | 8.7 |
| | 250 | 99.8 | 6.0 |

| Sample | Con. (ng/mL) | Recovery (%) | RSD (%) |
|---|---|---|---|
| Amitriptyline | 5 | 83.7 | 7.0 |
| | 10 | 81.8 | 2.8 |
| | 50 | 83.8 | 3.0 |
| | 250 | 88.4 | 2.7 |
| Sulpiride | 5 | 97.9 | 9.0 |
| | 10 | 95.5 | 8.5 |
| | 50 | 90.8 | 2.6 |
| | 250 | 92.6 | 3.0 |

# Application with MonoSpin® C18

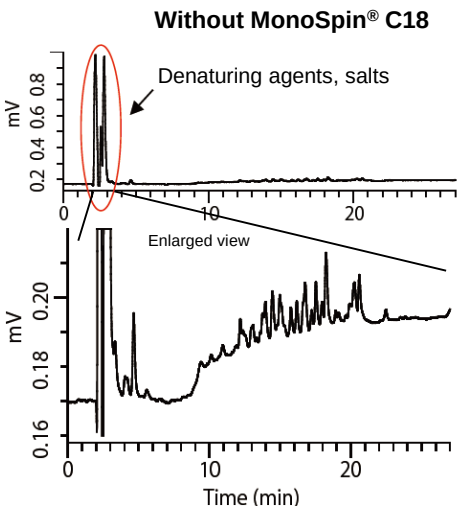
## Protein digests Using MonoSpin® C18

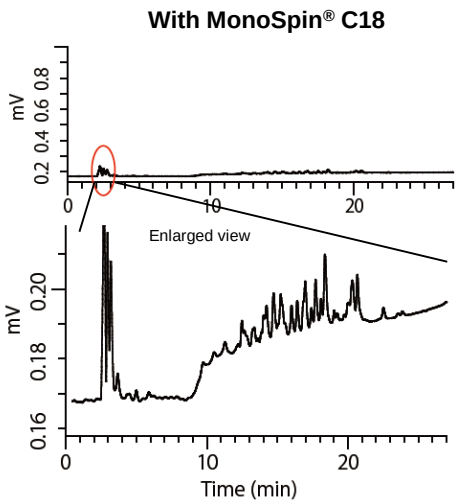
**Centrifuge** : 2,300 × *g*




| Sample preparation | Conditioning | Adsorption | Rinsing | Elution | Purified Sample |
|---|---|---|---|---|---|
| Prepare 800 µL sample solution. Add TFA to tryptic digest sample and adjust the TFA final concentration to 0.1 %. | Add 200 µL acetonitrile<br>↓ Centrifuge<br>Add 200 µL 0.1 %TFA | Add 800 µL sample solution | Add 200 µL 0.1 %TFA | Add 200 µL 60 % acetonitrile | |

**MonoSpin® C18 provide highly effective removal of concentrated denaturing agents and salts in tryptic digest samples**



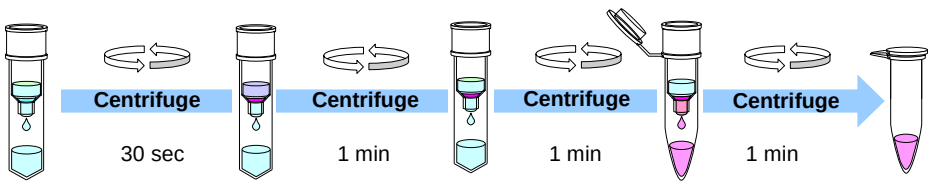




**Conditions**

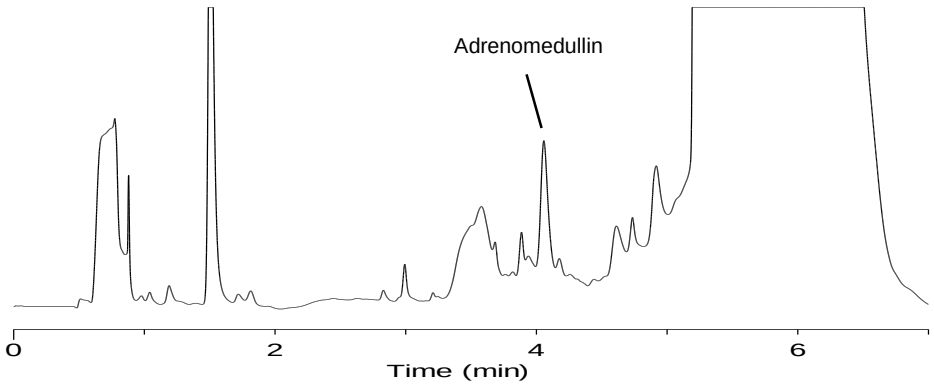
| | |
|---|---|
| Column | : Inertsil ODS-3 (3 µm, 150× 2.1 mm I.D.) |
| Eluent | : A) $H_2O$ (0.1 % TFA)<br>B) Acetonitrile (0.1 % TFA)<br>A/B = 90/10 - 20 min - 50/50 |
| Flow Rate | : UV 210 nm |
| Col. Temp. | : 0.2 mL/min |
| Detection | : 40 ℃ |
| Sample | : Digested BSA 2 µL |

## Recovery of Hormone in Serum Using MonoSpin® C18

**Centrifuge** : 2,300 × *g*




| Sample preparation | Conditioning | Adsorption | Rinsing | Elution | Purified Sample |
|---|---|---|---|---|---|
| Add 20 µL of 1 mg/mL of adrenomedullin to 190 µL serum. Add 0.1 %TFA to the serum solution and centrifuged at 10,000 × *g* for 1 min. Take the supernatant. | Add 200 µL acetonitrile<br>↓ Centrifuge<br>Add 200 µL 0.1 %TFA | Add 400 µL sample solution | Add 200 µL 0.1 % TFA | Add 200 µL 0.1 %TFA in 60 % acetonitrile | |




Conditions

| | |
|---|---|
| Column | : InertSustain C18 ( 2 µm, 50 × 2.1 mm I.D.) |
| Eluent | : A) 0.1 % TFA in $H_2O$<br>B) 0.1 % TFA in Acetonitrile<br>A/B = 85/15 – 5 min – 50/50 –2 min–50/50 |
| Flow Rate | : 200 µL/min |
| Col. Temp. | : 40 ℃ |
| Detection | : UV 210 nm |
| Injection Vol. | : 10 µL |

# Application with MonoSpin® C18 FF

## MonoSpin® C18 FF is ideal for high viscosity samples, such as whole blood and complex matrix samples.

**MonoSpin® C18 FF Specification**

| | |
|---|---|
| Through-pore | 20 µm |
| Meso-pore | 15 nm |
| Column size | Φ4.2 x 1.5 mm |
| Sample volume | 50 - 800 µL |
| Elution volume | 50 - 800 µL |
| Centrifugation speed | 1,000 x *g* |
| Maximum loading volume | 50 µg (Amitriptyline) |

### Relationship between centrifugation speed and flow of samples.

MonoSpin® C18 offer fast flow of viscosity samples at a low centrifugation speed (1,000 x g).

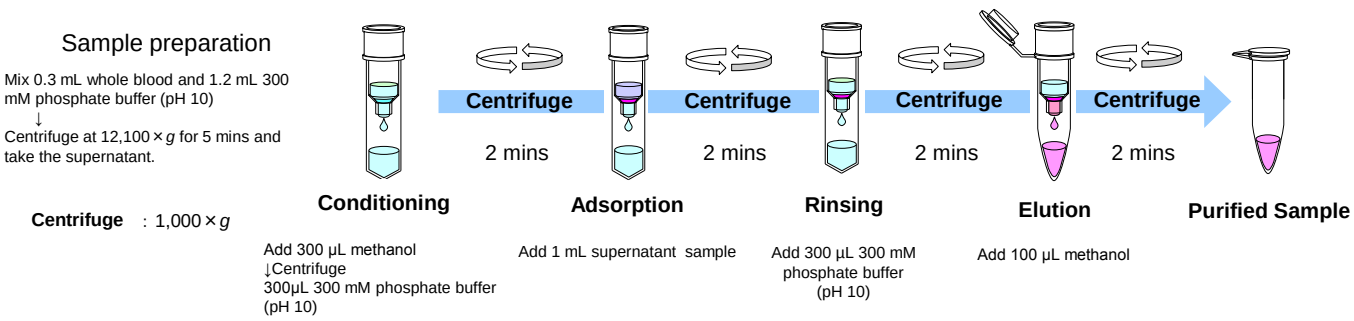
Comparison of each solution passing through MonoSpin® C18 and MonoSpin® C18 FF.

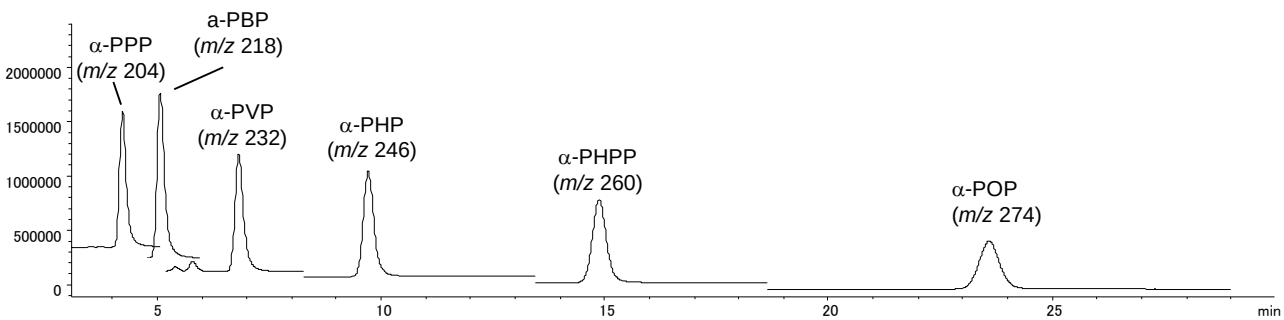
| Solvents | Volume | MonoSpin® C18* | MonoSpin® C18 FF |
|---|---|---|---|
| Methanol | 500 µL | ○ | ○ |
| Water | 500 µL | 400 µL | ○ |
| Serum** | 500 µL | 300 µL | ○ |

Conditions
Centrifuge at 1,000 ×*g* for 30 sec

* Generally, MonoSpin® C18 should be used with a centrifugation speed at 2,300 x g or higher for at least 30 sec.
** A supernatant from serum sample was used, which was centrifuged at 10,000 ×g for 1 min.

## Purification of Whole Blood Using MonoSpin® C18 FF



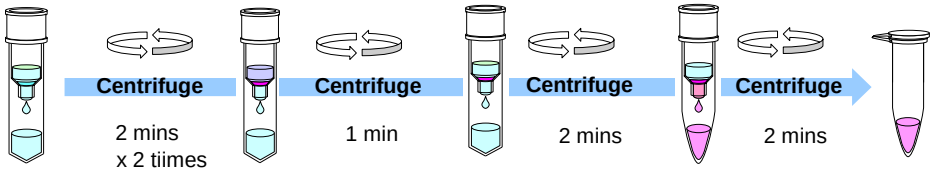

Conditions
Column : InertSustain Phenyl (3 µm, 150 ×2.1 mm I.D.)
Eluent : Acetonitrile-$HCOONH_4$(10 mM, 0.1 % HCOOH) = 25:75 (v/v)
Flow Rate : 0.2 mL/min
Col. Temp. : 40 ℃
Detection : MS(ESI)

# Application with MonoSpin® NH2

## Purification of Pyridylaminated(PA) Sugar Chain Using MonoSpin® NH2

Sample preparation

Prepare 800 µL sample solution. Add acetonitrile to PA sugar chain sample and adjust the acetonitrile final concentration from 90 to 95 %.




**Centrifuge** : 2,300 × *g*

**Conditioning**

Add 500 µL solution mixed with 250 µL 0.1% formic acid* in water and 250 µL 0.1% formic acid in acetonitrile.
↓Centrifuge
Add 500 µL solution mixed with 50 µL 0.1% formic acid in water and 450 µL 0.1% formic acid in acetonitrile.

**Adsorption**

Add 800 µL sample solution.

**Rinsing**

Add 500 µL solution mixed with 50 µL 0.1% formic acid in water and 450 µL 0.1% formic acid in acetonitrile.

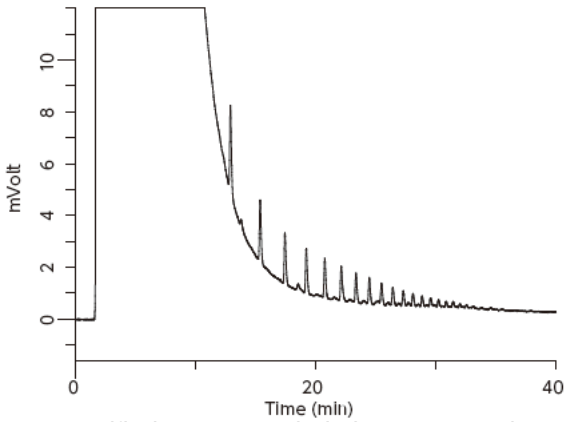
**Elution**

Add 50 - 800 µL mixed with 25 – 400 µL 0.1 % formic acid in acetonitrile and 25 – 400 µL water.

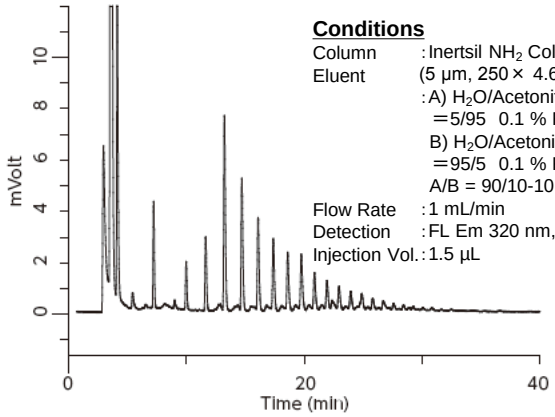
**Purified Sample**

*Acetic acid or TFA can also be used as an alternative to formic acid.

Without MonoSpin® NH2




With MonoSpin® NH2




**Conditions**

Column : Inertsil $NH_2$ Column (5 µm, 250 × 4.6 mm I.D.)
Eluent : A) $H_2O$/Acetonitrile =5/95 0.1 % Formic acid
B) $H_2O$/Acetonitrile =95/5 0.1 % Formic acid
A/B = 90/10-10 min–90/10-40 min-60/40
Flow Rate : 1 mL/min
Detection : FL Em 320 nm, Ex 400 nm
Injection Vol. : 1.5 µL

Purified PA sugar chain by HILIC mode.
MonoSpin® NH2 also can remove residual fluorescent labeling reagents.

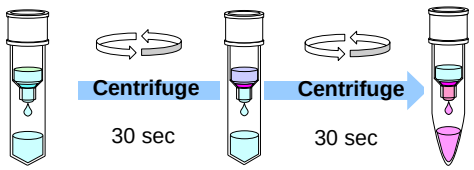
# Application with MonoSpin® SCX

## Fractionation Protein Digests Using MonoSpin® SCX

MonoSpin® SCX provide a fast and easy fractionation of peptides by stepwise Elution using buffers with different salt concentration.

Sample preparation

Prepare 500 µL peptide solution. First, desalt the peptide solution using MonoSpin C18. Then dissolve it with 0.1 formic acid.




**Centrifuge** : 10,000 × *g*

**Conditioning**

Add 300 µL 0.1 % formic acid.

**Adsorption**

Add 500 µL peptide solution.

**Elution**

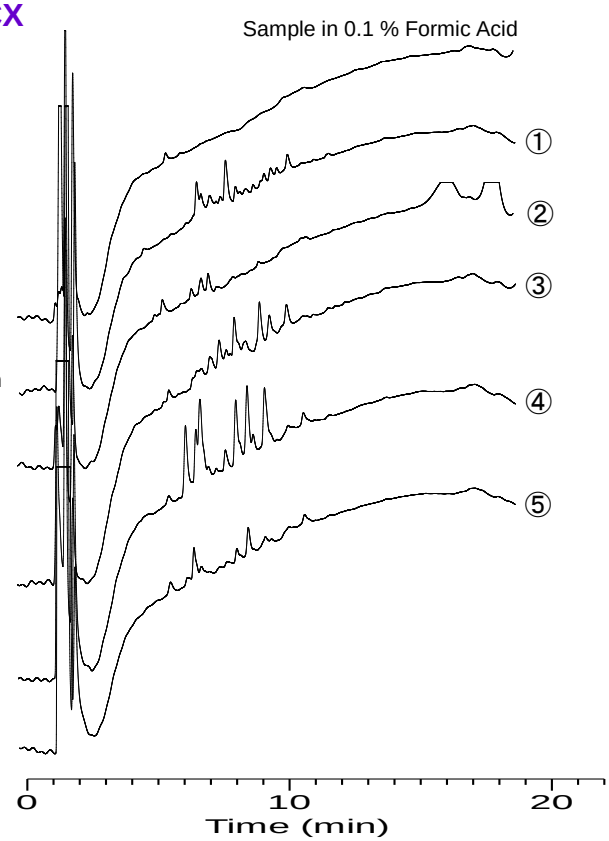
Always replace and attach a new recovery tube whenever adding a new elution buffer.

① 25 mM $HCOONH_4$ in 10% acetonitrile: 200 µL
② 50 mM $HCOONH_4$ in 10% acetonitrile: 200 µL
③ 100 mM $HCOONH_4$ in 10% acetonitrile: 200 µL
④ 500 mM $HCOONH_4$ in 10% acetonitrile: 200 µL
⑤ 1 M $HCOONH_4$ in 10% acetonitrile: 200 µL

**Conditions**

Column : Inertsil ODS-3 (3 µm, 2.1 × 150 mm)
Eluent A) $H_2O$ (0.1 % HCOOH)
B) Acetonitrile (0.1 % HCOOH)
A/B = 90/10 - 20 min - 50/50

Detection : UV 210 nm
Flow Rate : 0.2 mL/min
Col. Temp. : 40 ℃
Injection Vol. : 2 µL



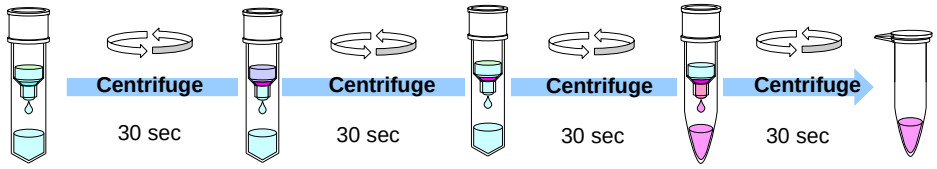
# Application with MonoSpin® CBA

## Purification of Paraquat and Diquat Using MonoSpin® CBA

Sample preparation

Prepare 600 µL sample solution mixed with 200 µL urine and 400 µL 10 mM potassium phosphate buffer (pH 7.0).

**Centrifuge** : 10,000 × *g*




**Conditioning**
Add 200 µL 10 mM potassium phosphate buffer (pH 7.0).

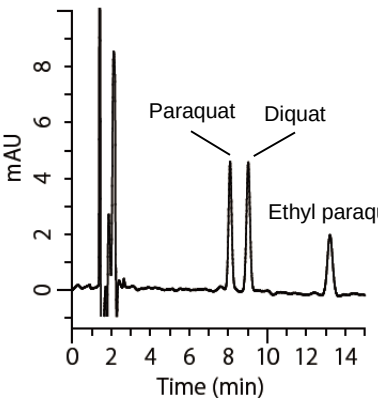
**Adsorption**
Add 600 µL sample solution.

**Rinsing**
Add 200 µL 10 mM potassium phosphate (pH 7.0).

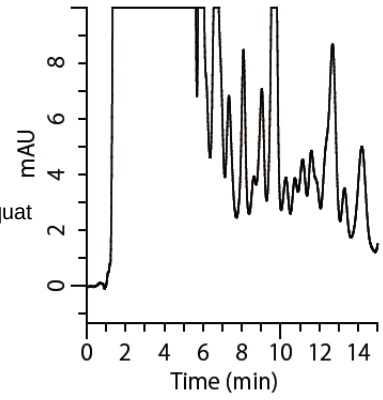
**Elution**
Add 200 µL solution mixed with 1 % HCL, 30 % methanol and 69 % water.

**Purified Sample**

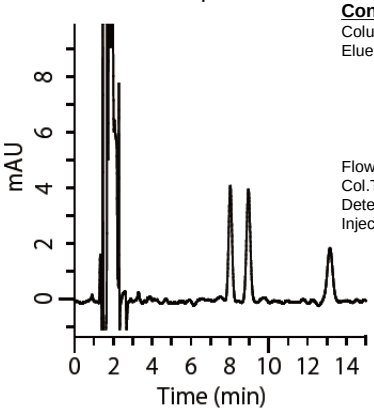
Standard Solution ( 1 µg/mL)




Urine + Pesticide ( each 1 µg/mL )




With MonoSpin® CBA




**Conditions**

| | |
|---|---|
| Column | : Inertsil ODS-3 (5 µm, 150 mm × 4.6 mm I.D.) |
| Eluent | : 0.2 M phosphoric acid, 0.1 M diethyl amine, 7.5 mM IPCC08(IPCC-0.8, Sodium 1-Octanesulfonate) /Acetonitrile=89/11 |
| Flow Rate | : 1 mL/min |
| Col.Temp. | : 40 ℃ |
| Detection | : PDA 290 nm |
| Injection Vol. | : 50 µL |

MonoSpin® CBA deliver highly efficient purification of strong basic pesticides such as paraquat and diquat.

# Application with MonoSpin® Trypsin

## Rapid Digestion of BSA using MonoSpin® Trypsin

Protein Digestion Time: 10 min

Protein Digestion Temperature: Room Temperature

Example of Reduction and Alkylation Protocol

1 mg Bovine serum albumin

----- Add 175 µL 500 mM Tris-HCL(pH 8.0) and 8 M urea ( Solution 1).
----- Add 25 µL 40 mg/mL dithiothreitol in solution 1.
-- Incubation* at 37 ℃ for 90 mins.

----- Add 50 µL 40 mg/mL iodoacetoamide in solution 1.
----- Incubation* at 37 ℃ for 30 mins without exposure to light.

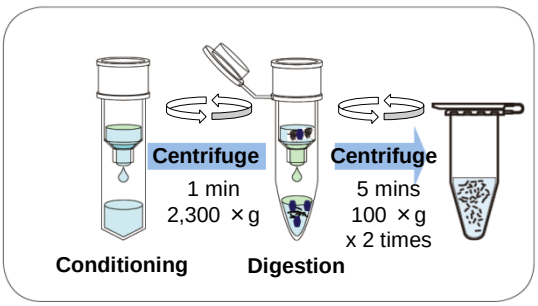
250 µL Reduced and alkylated protein

----- Add 50 mM ammonium bicarbonate to make the urea final concentration to 2 M and dilute it to 750 µL

* With gentle mixing

**MonoSpin® Trypsin**

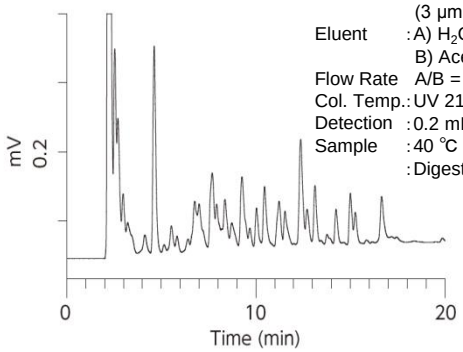
The protocol above is just an example. Optimize the protocol of preparation of reduced and alkylated sample depending on the types of proteins.



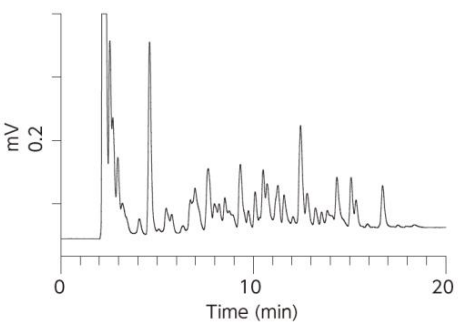

● In-solution protein digestion at 37 ℃, 10 hours

**Conditions**

| | |
|---|---|
| Column | : Inertsil ODS-3 (3 µm, 150 × 2.1 mm I.D.) |
| Eluent | : A) $H_2O$ (0.1 % HCOOH) B) Acetonitrile (0.1 % HCOOH) |
| Flow Rate | A/B = 90/10 - 20 min - 50/50 |
| Col. Temp. | : UV 210 nm |
| Detection | : 0.2 mL/min |
| Sample | : 40 ℃ |
| | : Digested BSA 2 µL |




● With MonoSpin Trypsin, Protein digestion at 25 ℃ for 10 mins



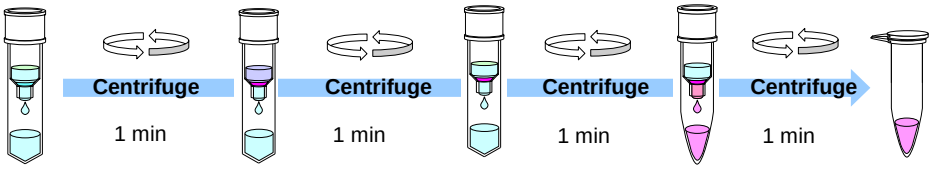
# Application with MonoSpin® PBA

## Purification of Catecholamine Using MonoSpin® PBA

### Sample preparation

Prepare 250 µL sample solution mixed with 200 µL urea or serum and 50 µL 1 M KH2PO4 adjusted the pH 8.0 using phosphoric acid

**Centrifuge** : 10,000 × *g*




**Conditioning**

Add 200 µL 1 % acetic acid
↓ Centrifuge
Add 200 µL 100 mM KH2PO4 adjusted the pH 8.0 using phosphoric acid

**Adsorption**

Add 250 µL sample solution

**Rinsing**

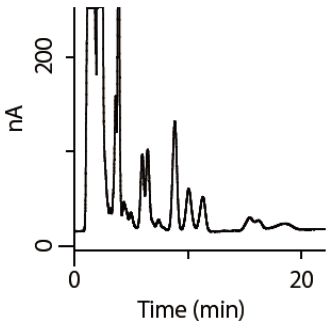
Add 200 µL 100 mM KH2PO4 adjusted the pH 8.0 using phosphoric acid

**Elution**

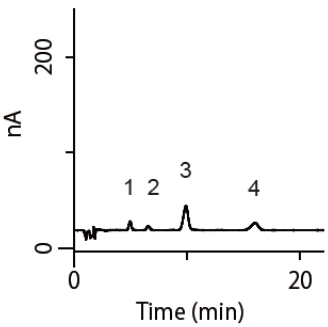
Add 200 µL 1 % acetic acid

**Purified Sample**

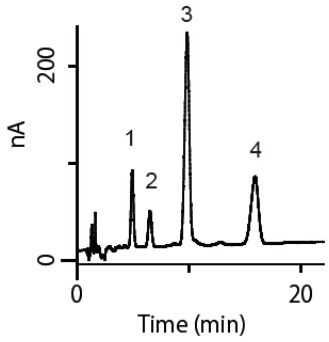
Without MonoSpin® PBA




500 µL elution using MonoSpin® PBA




50 µL elution using MonoSpin® PBA




**Conditions**

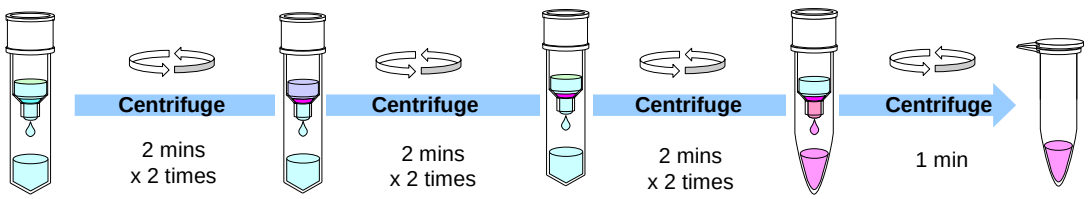
| | |
|---|---|
| Column | : Inertsil ODS-3 (5 µm, 150 mm × 2.1 mm I.D.) |
| Eluent | : 50 mM Phosphate buffer (pH 5.6)<br>50 mg/L EDTA<br>600 mg/L IPCC-008<br>-10 % Methanol |
| Flow Rate | : 0.3 mL/min |
| Col.Temp. | : 35 ℃ |
| Injection | : 5 µL |
| Detection | : ECD Pulse Mode |
| Sample | : 1. Noradrenaline<br>2. Adrenaline<br>3. DHBA<br>4. Dopamine |

MonoSpin® PBA can offer selective enrichment of catecholamines including compounds containing cis-diol.

# Application with MonoSpin® TiO

## Purification of Organic Phosphorous Pesticides in Serum Using MonoSpin® TiO

**Centrifuge** : 5,200 × *g*




**Conditioning**

Add 20 µL solution mixed with 16 µL 0.1 % formic acid in acetonitrile and 4 µL 0.1 % formic acid in water.
↓ Centrifuge
Add 20 µL solution mixed 10 µL 0.1 % formic acid in acetonitrile and 10 µL 0.1 % formic acid in water.

**Adsorption**

Add 50 µL sample solution mixed with 10 µL sample and 40 µL water.
↓ Centrifuge
Collect the eluate from the recovery tube and attach a new one. Then apply the eluate to MonoSpin® TiO again.

**Rinsing**

Add 20 µL solution mixed with 10 µL 0.1 % formic acid in acetonitrile and 10 µL 0.1 % formic acid in water.
↓ Centrifuge
Repeat the above protocol.

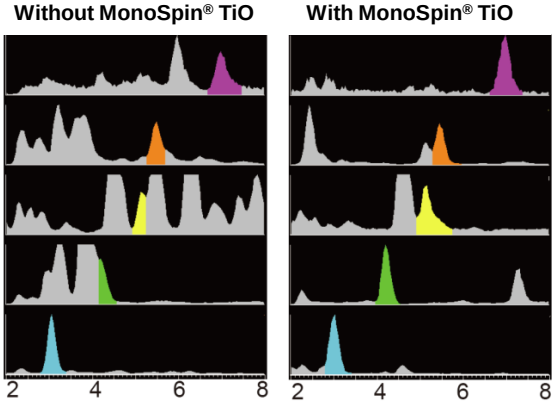
**Elution**

Add 50 µL 2 % NH3.

**Purified Sample**

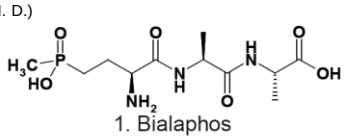
Derivatize the purified sample with N-Acetyl-O-methylate and then inject to LC/MS/MS.

**Without MonoSpin® TiO** **With MonoSpin® TiO**

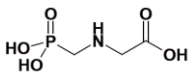



**Conditions**

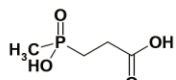
| | |
|---|---|
| Column | : ODS Column (150 × 2.1 mml. D.) |
| Eluent | : CH3OH<br>20 mM HCOONH4 (pH 3.0)<br>A/B = 15/85, w/w |
| Flow Rate | : 200 µL/min |
| Detection | : SIM |
| Injection Vol. | : 5 µL |
| Sample | : 1. Bialaphos<br>2. Glyphosate<br>3. MPPA<br>4. Glufosinate<br>5. AMPA |



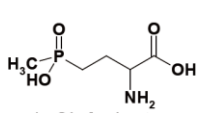
1. Bialaphos



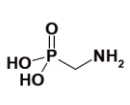
2. Glyphosate



3. MPPA



4. Glufosinate



5. AMPA

# References of Application

| Product | Target compounds | Sample matrix | Concentration | Recovery rate | Detector | Reference No. |
|---|---|---|---|---|---|---|
| MonoSpin® C18 | amitraz, metabolites | serum | 5 ng/mL | 95.5, 92.2 % | LC-MS | 1 |
| | dibudcaine, naphazoline | serum | 5 - 10 ng/mL | 70.2 - 78.6 % | LC-MS | 2 |
| | MA, AP, MDA, MDMA | urine | 100 ng/mL | 96 - 111 % | LC-UV | 3 |
| | 9 cold medicines | serum | 5 - 50 ng/mL | 2.5 - 73.8 % | GC-MS | 4 |
| | amphetamines (AP, MA, MDA, MDMA) | urine | 5 - 10 ng/mL | 84 - 94 % | GC-MS | 5 |
| | eperison | serum | 0.5 ng/mL | 92.8 - 96.0 % | GC-MS | 6 |
| | paraquat, diquat, fenitrothion | serum, urine | 25 - 100 ng/mL | 51.3 - 106.1 % | GC-MS | 7 |
| | arsenics | urine | 1 ng/mL | 91.9 - 106.5 % | GC-MS | 8 |
| | MAM-2201 | blood | 1 ng/mL | - | LC-MS/MS | 9 |
| | a-PVP, a-PBP | urine | 1 ng/mL | 82 - 100 % | GC-MS | 10 |
| | a-PVP, a-PBP | hair | 0.2 ng/mL | 75.5 - 101.5 % | LC-MS | 11 |
| | Phthalic acid esters | physiological saline | 0.2 - 50 µg/L | 71.2 - 107.3 % | - | 12 |
| | ＜desalting＞ | digested peptides | - | - | - | 13 |
| | ＜desalting＞ | iTRAQ labeled samples | - | - | - | 14 |
| | MAM-2201 | blood | 2.5 - 100 ng/mL | 1 ng/mL | - | 15 |
| | Naringin | grapefruit juice | 10 - 500 µM | 10 µM | - | 16 |
| MonoSpin® SCX | opiates<br>benzodiazepines, metabolites | urine<br>serum | 10 ng/mL<br>1 - 10 ng/mL | 69.2 - 98.9 %<br>83.3 - 112.3 % | LC-MS | 17 |
| | ＜Pre-column fluorescence derivatization＞ | - | - | - | - | 18 |
| | ＜desalting of amino acid＞ | - | - | - | - | 19 |
| MonoSpin® C18-CX | acidic and basic drugs | urine | 1 - 25 ng/mL | 65 - 123 % | GC-MS | 20 |
| | ＜halogenated compounds＞ | cells | - | - | - | 21 |
| MonoSpin® C18-AX | amphetamines (AP, MA), opiates, THC | urine | 2 - 10 ng/mL | 93.1 - 108.1 % | GC-MS | 22 |
| MonoSpin® PBA | Adenosine | urine | 6 µM | 80 - 113 % | - | 23 |

## References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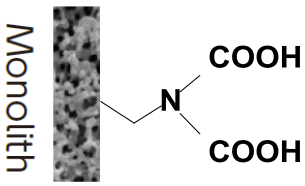
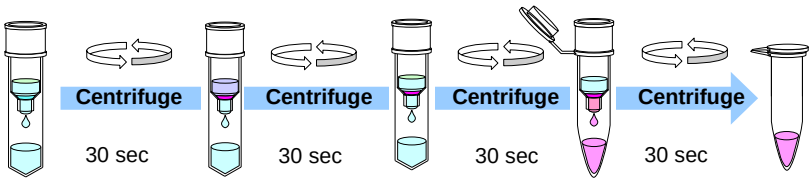
[21] Toxicology., 314 (2013) 22-29
[22] Forensic Toxicol., 31 (2013) 312-321.
[23] Biosensors and Bioelectronics., 41 (2013) 379-385

# Application with MonoSpin® ME




- Bonded with iminodiacetic acid.
- Optical for the recovery and purification of metal ions.
- Specifically, appropriate for the extraction and purification of trace Pb in blood or urine.
- Exellent for removing inorganic divalent cations from sample to prevent ion suppression for LC/MS/MS applications.

## Recovery of Metal Ions Using MonoSpin® ME




**Conditioning**
Add 200 µL water
↓ Centrifuge
Add 200 µL 2N-$HNO_3$
↓ Centrifuge
Add 400µL 100 mM $CH_3COONH_4$ (pH 5.5)

**Adsorption**
Add 500 µL 25µg/mL $Cu^{2+}$

**Rinsing**
Add 10 mM $CH_3COONH_4$ (pH 5.5)

**Elution**
Add 500 µL 2N-$HNO_3$

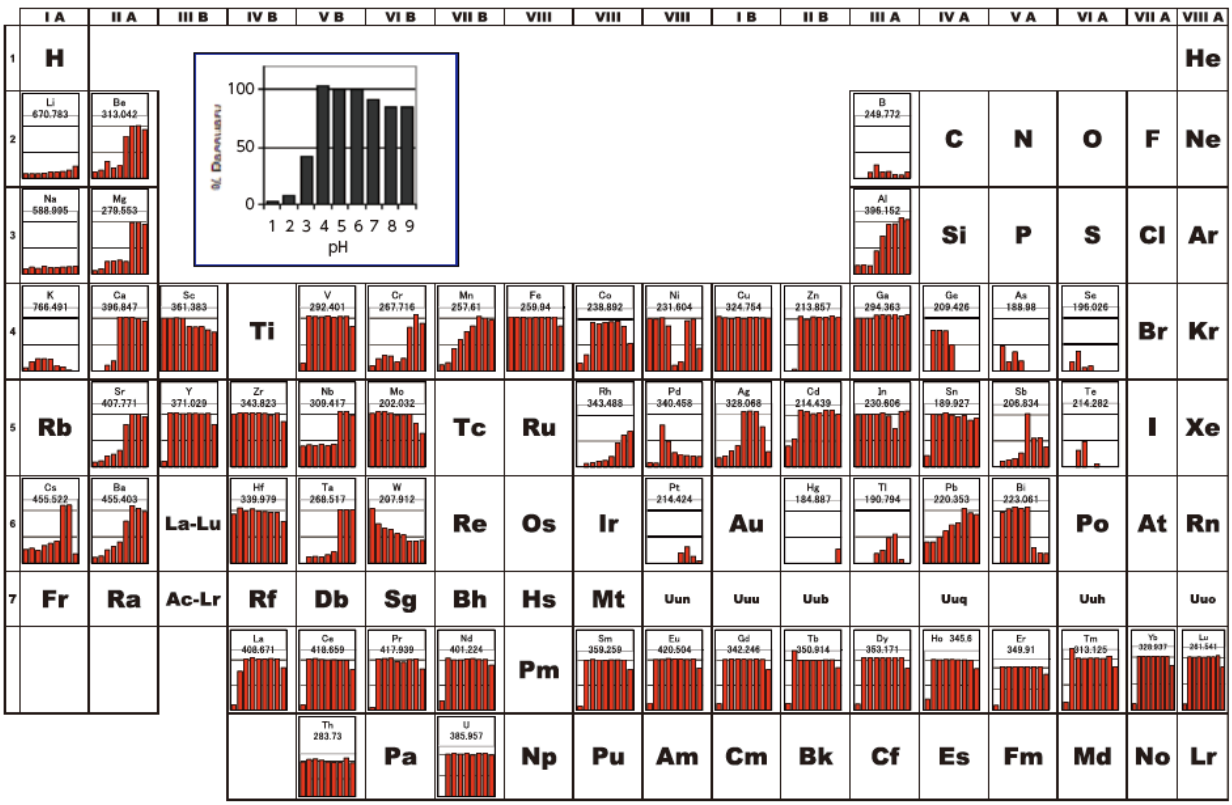
**Purified sample**

**Centrifuge** : 3,000 × *g*

Recovery rate of $Cu^{2+}$ using Zeeman GF-A-AF system

| Number of Injections | Volume of solvent introduced (mL) | Recovery rate (%) |
|---|---|---|
| 1 | 0.8 | 98±4 |
| 2 | 1.6 | 97±5 |
| 3 | 2.4 | 95±5 |
| 4 | 3.2 | 95±5 |
| 5 | 4 | 94±3 |

## Retention characteristics of Metal Element Using Iminodiacetic Acid Functional Groups with Various pH.




* Do not ensure the quality of MonoSpin® ME on the above table.

# Application with MonoSpin® Phospholipid

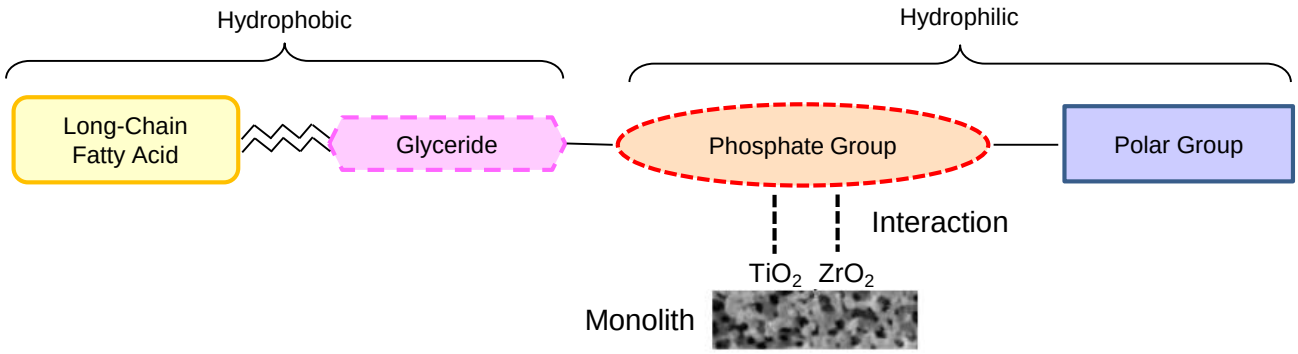
## Features


- Simple & easy protocol to remove phospholipids from biological samples.
- Removes more than 90% of phospholipids resulting in eliminating ion suppression.
- Also removes phospholipids from a serum sample volume of 50 μL.

## Retention Mechanism of Phospholipids

- Monolith skeletal structure coated with $TiO_2$ and $ZrO_2$.
- Selectively interacts with metal oxides and phosphorylated compounds, resulting in removing more than 90 % of phospholipids.

### Structure of Phospholipids

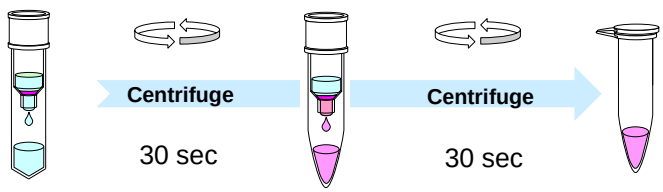



## Protocol of removal of phospholipids

Sample preparation

Mix 0.1% formic acid in acetonitrile with serum ( 4 : 1 ) in 2 mL tube .
↓ Centrifuge at 10,000 ×*g* for 30 sec.
Take the supernatant.

**Centrifuge** : 3,000 ×*g*



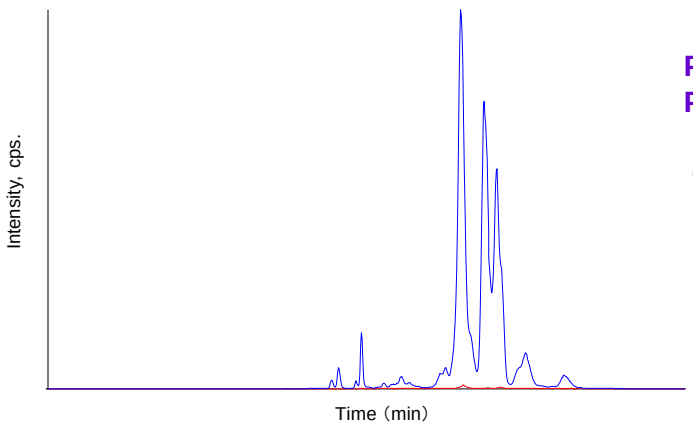

**Conditioning**

Add 200 μL 0.1 % formic acid in acetonitrile.

**Adsorption**

Add 50 μL sample solution

**Phospholipid-free sample**




### Phospholipid Removal Efficiency of MonoSpin® Phospholipid

Deproteinized and centrifuged supernatant

Sample clean up by MonoSpin® Phospholipid ( Removes more than 90% of phospholipids )

Related Product : ***FastRemover® for Phospholipid***

The FastRemover® for Phospholipid 96-well plate deliver a rapid and effective removal of proteins and phospholipids in plasma and serum samples without sacrificing the recovery of your target analytes.

# Rapid Purification of Antibodies

## MonoSpin® ProA, MonoSpin® ProG

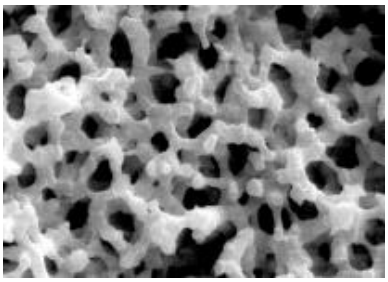
MonoSpin® ProA and MonoSpin® ProG are available already immobilized onto a silica monolith offering rapid purification of antibodies. A 96-well plate format is available to purify a number of analyte.


Spin columns

96 well plate

### Features

The silica is modified with a hydrophilic polymer and then immobilized with either Protein A or Protein G to prevent the adsorption of proteins, resulting in higher purification and recovery of antibodies.



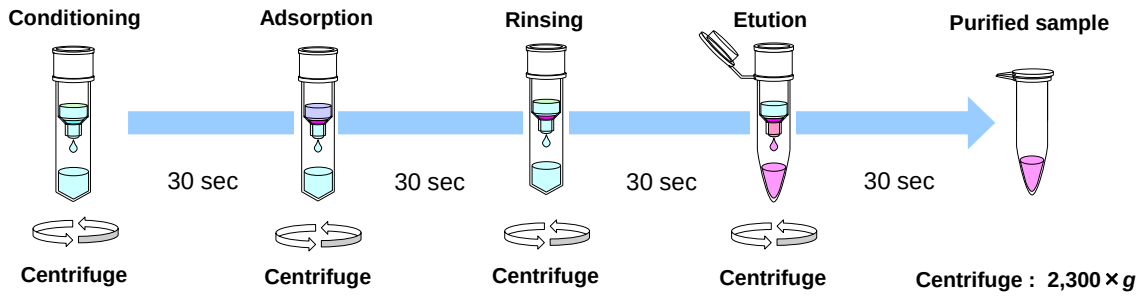
| Specification | |
|---|---|
| Bonded phase | Protein A or Protein G |
| Through-pore size | 2 µm |
| Meso-pore size | 60 nm |
| Column size | Φ4.6 x 1.5 mm |
| Sample volume | 50 - 500 µL |
| Recovery rate | MonoSpin ProA：IgG 90 %（With 400 µg IgG） |
| | MonoSpin ProG：IgG 90 %（With 300 µg IgG） |
| Elution volume | 50 µL |
| Centrifugation speed | 2,300 × *g* |

### Antibody compatibility table

| Species | Antibody class | Protein A | Protein G |
|---|---|---|---|
| Human | IgG | ◎ | ◎ |
| | IgG1 | ◎ | ◎ |
| | IgG2 | ◎ | ◎ |
| | IgG3 | — | ◎ |
| | IgG4 | ◎ | ◎ |
| | IgM | — | — |
| | IgA | — | — |
| | IgE | — | — |
| | IgD | — | — |
| | Fab | ○ | ○ |
| | ScFv | ○ | — |

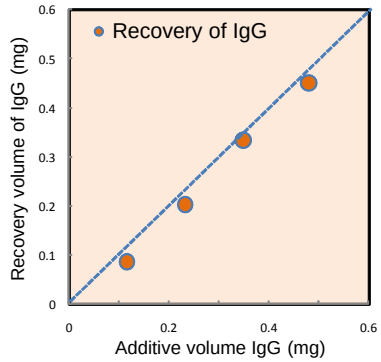
# Rapid Purification of Antibodies

## Purification of IgG Using MonoSpin® ProA and MonoSpin® ProG in Only 5 min.

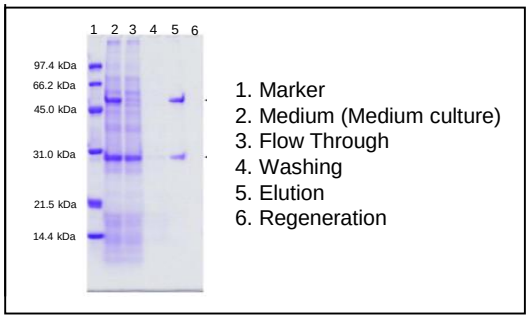



As shown below, the antibody concentrations were determined quantitatively from medium of CHO cells. The purified antibodies show very less impurities by the results from electrophoresis.

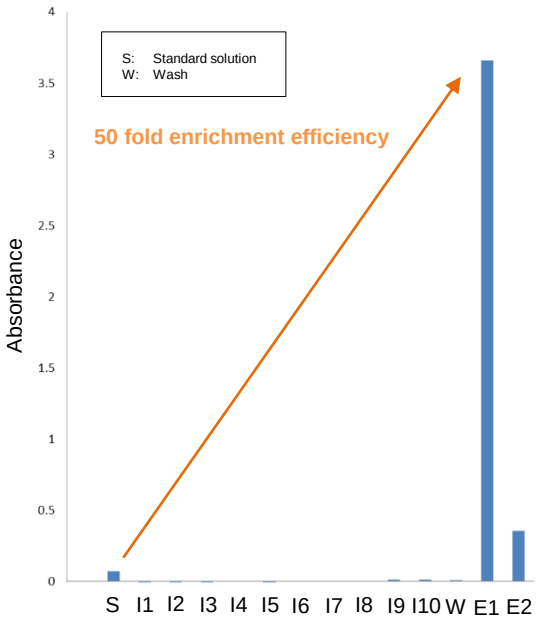
**Calibration curve of IgG concentration**




**Results of recovery electrophoresis**




## Enrichment of Antibody Solution Using MonoSpin® ProA




500 μL volume of 0.025 mg / mL of human IgG solution is applied to MonoSpin® ProG spin column ( In = I1 – I10 ). And then the elution of IgG concentration is measured with 100 μL elution buffer twice (En = E1 and E2). The first IgG elution (E1) is 50 fold concentration of standard solution and indicates 90 % recovery of IgG without the loss of IgG.

Number of injections (In) or Elution (En) ( Times:n=1,2,・・・, 10 ).

# Rapid Purification of Antibodies

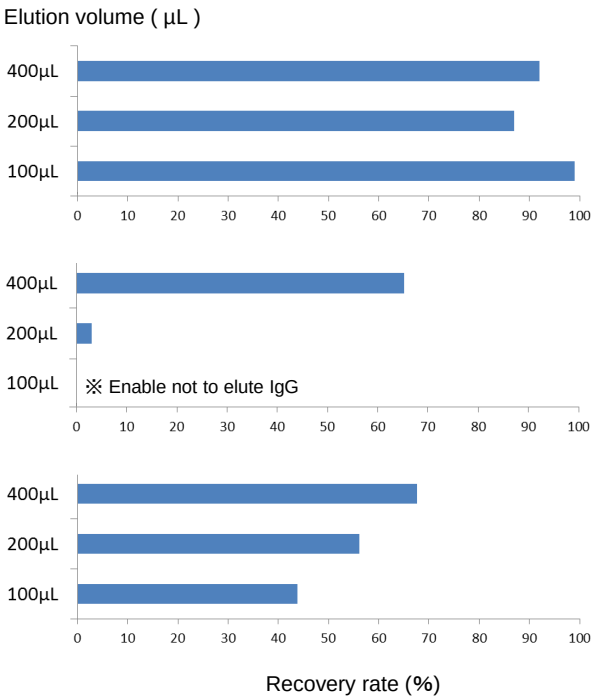
## Relationship Between Elution Volume and Recovery Rate With Other Brands Products.

MonoSpin ® ProA only requires 100 µL elution buffer, providing a recovery rate of at least 90% IgG. On the other hand, other brands products requires 400 µL of elution buffer with a recovery rate of 70% IgG.

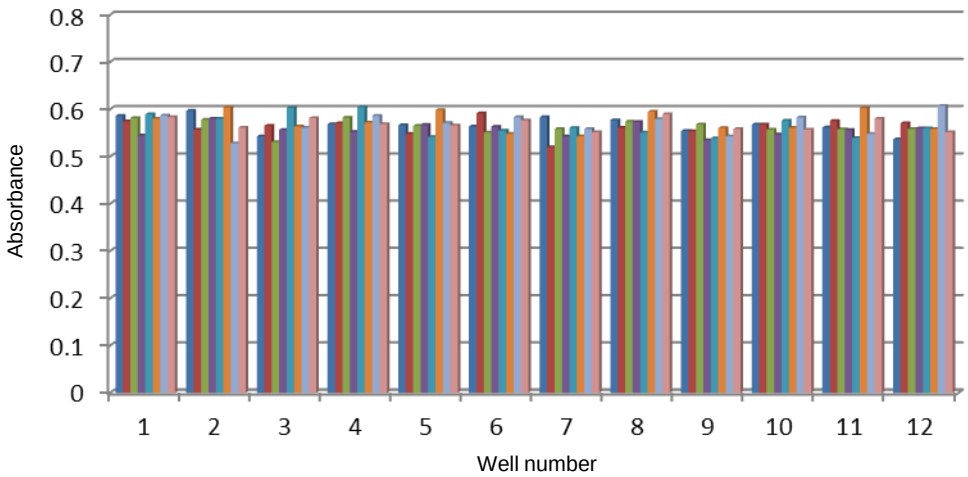
**MonoSpin ® ProA**
90 % recovery rate of IgG with 100 µL elution.

**Brand T's Product**
60-65 % recovery rate of IgG with 400 µL elution.

**Brand G's Product**
65-70 % recovery rate of IgG with 400 µL elution.




## Recovery Rate and Reproducibility of IgG from medium cultured CHO cells with MonoSpin® ProA 96 Well Plate




| | |
|---|---|
| Sample volume: | 150 µL |
| Elution volume: | 150 µL |
| Recovery rate: | 90 % (CV 3.1 %) |
| IgG concentration: | 1.3 mg/mL |

# MonoSpin® Series Ordering Information

## MonoSpin® Column Small Type*

| Products | Quantity | Cat.No. |
|---|---|---|
| MonoSpin® C18 | 50 pcs | 5010-21700 |
| | 100 pcs | 5010-21701 |
| MonoSpin® C18 FF | 50 pcs | 5010-21670 |
| | 100 pcs | 5010-21671 |
| MonoSpin® Ph | 50 pcs | 5010-21733 |
| | 100 pcs | 5010-21734 |
| MonoSpin® C18-AX | 50 pcs | 5010-21735 |
| | 100 pcs | 5010-21736 |
| MonoSpin® C18-CX | 50 pcs | 5010-21731 |
| | 100 pcs | 5010-21732 |
| MonoSpin® SAX | 50 pcs | 5010-21720 |
| | 100 pcs | 5010-21721 |
| MonoSpin® SCX | 50 pcs | 5010-21725 |
| | 100 pcs | 5010-21726 |
| MonoSpin® NH2 | 50 pcs | 5010-21710 |
| | 100 pcs | 5010-21711 |
| MonoSpin® CBA | 50 pcs | 5010-21729 |
| | 100 pcs | 5010-21730 |
| MonoSpin® Amide | 50 pcs | 5010-21727 |
| | 100 pcs | 5010-21728 |
| MonoSpin® PBA | 50 pcs | 5010-21715 |
| | 100 pcs | 5010-21716 |
| MonoSpin® TiO | 50 pcs | 5010-21705 |
| | 100 pcs | 5010-21706 |
| MonoSpin® Trypsin | 50 pcs | 7820-11300 |
| | 100 pcs | 7820-11301 |
| MonoSpin® ME | 50 pcs | 5010-21737 |
| | 100 pcs | 5010-21738 |
| MonoSpin® Phospholipid | 50 pcs | 5010-21698 |
| | 100 pcs | 5010-21699 |

* Each MonoSpin® products are attached with 1.7 mL recovery and 2.0 mL waste tubes.

MonoSpin® Column Small type

1.7 mL Recovery Tube

2.0 mL Tube for Waste Solution

## MonoSpin® Column Large Type**

| Products | Quantity | Cat.No. |
|---|---|---|
| MonoSpin® L C18 | 30 pcs | 7510-11320 |
| MonoSpin® L SAX | 30 pcs | 7510-11321 |
| MonoSpin® L SCX | 30 pcs | 7510-11322 |
| MonoSpin® L NH2 | 30 pcs | 7510-11323 |
| MonoSpin® L CBA | 30 pcs | 7510-11324 |
| MonoSpin® L ME | 30 pcs | 7510-11325 |
| MonoSpin® L Phospholipid | 30 pcs | 7510-11326 |

MonoSpin® Column Large type

** MonoSpin® Large type columns dose not come with any recovery and waste tubes. Please prepare 50 mL centrifuge tubes separately.

# MonoSpin® Series Ordering Information

## MonoSpin® ProA, MonoSpin® ProG

| Products | Quantity | Cat.No. |
|---|---|---|
| MonoSpin® ProA Small type | 10 pcs | 7510-11310 |
| MonoSpin® ProG Small type | 10 pcs | 7510-11311 |
| MonoSpin® ProA 96-well plate | 1 pcs | 7510-11312 |
| MonoSpin® ProG 96-well plate | 1 pcs | 7510-11313 |

MonoSpin® Column Small type

96-well plate