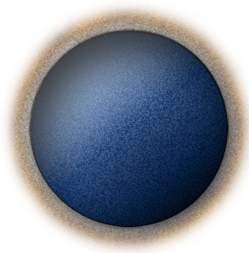


magtivio

MagSiMUS -TOX^{PREP} Type I

Art.No.
MD03028



Product Manual

Version 1.0 | 07/08/2018

MagSiMUS-TOX^{PREP} Type I

Sample preparation for LC-MS/MS based therapeutic drug monitoring

This product is for Research Use Only (RUO). Not for drug, household or other uses. For more information, please consult the appropriate Safety Data Sheet (SDS), available on our website at www.magtivio.com.

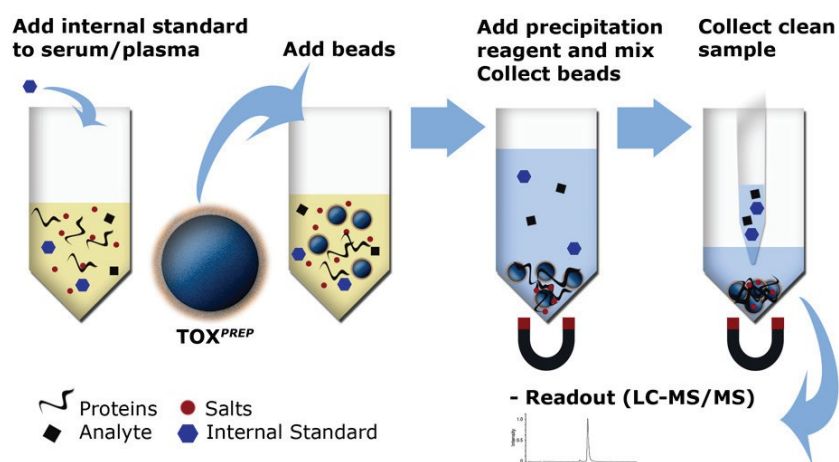
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1. General Information

MagSiMUS-TOX^{PREP} is a sample preparation and clean-up method for serum, plasma, whole blood and urine samples prior to LC-MS/MS-based analysis. The magnetic properties enable complete pellet formation even in viscous samples. Interfering compounds, such as proteins and phospholipids, are depleted while keeping analytical targets in suspension. This enables direct injection into an LC-MS/MS instrument, without the need for further purification (e.g. solid phase extraction).

The MagSiMUS-TOX^{PREP} process is outlined in the illustration below. The supernatant containing the analyte of interest is directly injected or transferred to a second tube, vial, microtiter plate or other suitable format for analysis.



Sample preparation principle: In the first step internal standard is added. By addition of MagSiMUS-TOX^{PREP} Type I Particle Mix and precipitation reagent proteins are bound to magnetic beads. The proteins are depleted from the reaction mix by simple magnetic separation. The purified sample can be directly injected into LC-MS/MS or can be stored for later analysis.

MagSiMUS-TOX^{PREP} is suitable for automation, providing a walk-away solution for sample preparation in formats like microtiter plates, microtubes or HPLC vials. Multiple parameters can be analyzed in a single microtiter plate (multi-parameter set-up) saving time and consumable costs.

2. Intended use

MagSiMUS-TOX^{PREP} is intended for clean-up of serum, plasma, whole blood or urine prior to LC-MS/MS analysis. The product is available in two versions: Type I and Type II. The Particle Mixes of MagSiMUS-TOX^{PREP} Type I and MagSiMUS-TOX^{PREP} Type II are suitable for different sets of parameters. The sample preparation method is the same for both products. Accessory products OPR I and OPR VI can be ordered separately.

Whole blood or urine samples can be analyzed as well. The required Lysis Buffer for whole blood (MD71630) and Urine Stabilization Buffer (MD71730) can be ordered separately.

The table below lists several examples of parameters suitable for MagSiMUS-TOX^{PREP} Type I sample preparation.

Parameter class	Examples of parameter
Drugs of Abuse	Ethyl glucuronide
Metabolites	Methylmalonic acid Folic acid Arachidonic acid
Vitamins	Vitamin K

For a comprehensive listing of >220 parameters please visit the magtivio webpage and download the **MagSiMUS Selection Guide** under Resources.

3. Materials

3.1 Kit contents

Description (MagSiMUS-TOX ^{PREP} Type I)	MD03028 (500 preps)
MagSiMUS-TOX ^{PREP} Type I Particle Mix	20 mL
Internal Standard Dilution Reagent A (ISDR A)	15 mL
Internal Standard Dilution Reagent B (ISDR B)	15 mL
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All reagents are supplied with an additional 10% volume to account for loss of reagents in the protocol.

3.2 Kit components available separately

Product	Volume	Article Number
OPR I Organic Precipitation Reagent I; for acetonitrile-based protein precipitation	100 mL	MD71130
OPR VI Organic Precipitation Reagent VI; for methanol-based protein precipitation, includes: - OPR II - PRZS I	104 mL 12.3 mL	MD71335
Internal Standard Dilution Reagent A (ISDR A)	100 mL	MDRE00110100
Internal Standard Dilution Reagent B (ISDR B)	100 mL	MD71530
Lysis Buffer for whole blood	100 mL	MD71630
Urine Stabilization Buffer	10 mL	MD71730

3.3 Materials supplied by the user

Reagents	
Internal Standard	Appropriate Internal Standard needs to be provided by the user

Consumables & Equipment

Protocol	Manual use	Automated use
Magnetic separator	MM-Separator M12 + 12 P (Art.No.: MDMG0001): Magnetic separator for 1.5 mL and 2 mL microtubes	MM-Separator 96 SBS BC (Art.No.: MDMG0007): Magnetic separator for bottom collection in 96-well V- and U-bottom microtiter plates
Sample containers	1.5 and 2 mL microtubes	MagSiMUS processing MTP (Art.No.: MDPL0014)
Micropipettes	10-100 µL, 20-200 µL and 100-1000 µL	20-200 µL

4. Kit Usage

4.1 Kit storage

- This product is stable for up to 2 years, but no longer than the expiry date on the label.
- Store the MagSiMUS-TOX^{PREP} Type I Particle Mix at 2-8°C in a well closed vial and in upright position to prevent drying. Do not freeze!

4.2 Safety

- When working with biological samples always wear a suitable lab coat and disposable gloves. Biological samples need to be considered as potentially hazardous.
- For more information, please consult the appropriate safety data sheets (SDS). These are available online at www.magtivio.com.

4.3 Preparation of Internal Standard solutions

- In the MagSiMUS-TOX^{PREP} clean-up protocol, 50 µL serum and 20 µL Internal Standard solution is used. The volume difference between sample and Internal Standard needs to be compensated by preparing the Internal Standard in a 2.5X concentrated solution.
- The Internal Standard working solution should contain >95% Internal Standard Dilution Reagent (ISDR A or ISDR B)
- Use ISDR A in conjunction with an OPR I protocol, and use ISDR B with a OPR VI protocol

4.4 Handling guidelines

- When transferring purified samples, inspect for carry-over of magnetic beads into the read-out vial, tube or plate. Beads can interfere with injection in LC-MS/MS instruments. If beads are present in final sample, perform the separation again.
- To prevent evaporation, buffer containers should be closed immediately after usage. After preparation the tubes, vials or microtiter plates containing purified samples should be closed or sealed immediately.
- MagSiMUS-TOX^{PREP} Type I is suitable for sample preparation in microtiter plate format using automated liquid handling devices. To prevent carry-over of magnetic beads into the purified sample, the magnetic beads need to be collected at the bottom of the microtiter plate. MM-Separator 96 SBS BC (Art.No. MDMG0007) provides efficient bottom collection.

4.5 Minimum requirements for LC-MS/MS

- Most commonly tested parameters can be directly analyzed after purification with MagSiMUS-TOX^{PREP} Type I.
- To prolong the lifetime of the analytical column in the LC-MS/MS work-flow the use of a guard column is highly recommended. Guard columns are available from different HPLC accessories suppliers and your mass spectrometry supplier. To prevent any particle carry over onto the guard column its additionally recommended to protect the guard column with a cap frit also available from different HPLC suppliers.
- Depending on the sensitivity of the read-out instrument and concentration of the parameter, additional sample concentration steps may be needed.

5. Protocols

5.1 Protocol selection

magtivio has developed different protocols for the removal of proteins and additional unfavorable compounds:

- 5.3 - Protocol for serum and plasma samples using acetonitrile-based precipitation reagent OPR I
- 5.4 - Protocol for serum and plasma samples using methanol-based precipitation reagent OPR VI
- 5.5 - Protocol for whole blood samples using methanol-based precipitation reagent OPR VI
- 5.6 - Protocol for urine samples using acetonitrile-based precipitation reagent OPR I
- 5.7 - Protocol for urine samples using methanol-based precipitation reagent OPR VI

The selection is based on the user preference for acetonitrile or methanol as precipitation agent with regard to the compatibility of the solvent to the LC-MS/MS setup used (mobile phases in the LC).

For further technical details and assistance, please contact magtivio technical support at support@magtivio.com.

5.2 Considerations for optimization

Samples processed with MagSiMUS protocols are characterized by a high content of methanol or acetonitrile. Besides that, processed serum and plasma samples are diluted by a factor 7.2, while whole blood is diluted by a factor 15.8. If desired, the processed samples can be diluted, or evaporated and reconstituted in mobile phase to improve injection conditions and compatibility to LC conditions. This may be particularly helpful in the process of replacing centrifugation-based methods such as protein crash or LLE.

The protocols are designed so that internal standard follows the exact same procedure as analytes in the sample. As a more practical and cost-friendly alternative, the internal standard may be added into the precipitation reagent. In that case, make sure to calculate the appropriate concentration for the internal standard in OPR I or OPR VI, and compensate the volume of missing ISDR A or ISDR B by increasing the volume of OPR I or OPR VI with 20 μ L.

For analytes requiring high measurement sensitivity, samples may need to be concentrated after processing with MagSiMUS. This can be done for instance by using a SPE or Trap column, but also by upscaling MagSiMUS protocols, followed by evaporation and reconstitution in a small solvent volume.

5.3 Protocol for serum and plasma samples using acetonitrile-based precipitation

Before use, vortex **MagSiMUS-TOX^{PREP} Type I Particle Mix** to fully resuspend the beads.

Make sure the Internal Standard solution is prepared as described in chapter 4.3.

Before use, allow products and samples to come to room temperature. Homogenize samples properly before starting the protocol.

1. Transfer 50 μL serum or plasma to a 2 mL microtube or microtiter plate
2. Add 20 μL Internal Standard solution to the sample
3. Add 40 μL MagSiMUS-TOX^{PREP} Type I Particle Mix and mix by pipetting
4. Add 250 μL OPR I and mix by 10 aspiration and dispensing cycles

This step is crucial for the performance of this kit. By the addition of precipitation reagent to the reaction mix, precipitated proteins and the bead mix form a voluminous aggregate which needs to be dispersed in a homogeneous suspension by pipetting

5. Place the samples on a magnetic separator and wait for 2 minutes until the supernatant is clear. For tubes use MM-Separator M12 + 12 P and for microtiter plates use MM-Separator 96 SBS BC
6. While avoiding contact with the pellet, transfer up to 80 μL of the supernatant to a new microtube, microtiter plate or HPLC vial for injection. Seal/close sample container to avoid evaporation and contamination

Make sure that no beads or parts of the pellet are transferred to the new sample container

5.4 Protocol for serum and plasma samples using methanol-based precipitation

Before use, vortex **MagSiMUS-TOX^{PREP} Type I Particle Mix** to fully resuspend the beads.

Make sure the Internal Standard solution is prepared as described in chapter 4.3.

Before use, allow products and samples to come to room temperature. Homogenize samples properly before starting the protocol.

Prepare Organic Precipitation Reagent VI (OPR VI)

- Add the content of PRZS I to the bottle of Organic Precipitation Reagent II and mix by inverting 5 times.
- Store prepared OPR VI at 2-8°C for no longer than 7 days.

Alternatively, prepare OPR VI with PRZS I and Organic Precipitation Reagent II for a specific number of samples (N):

- Add (N+1)***27.5 µL PRZS I** to (N+1)***232.5 µL OPR II**

1. Transfer 50 µL serum or plasma to a 2 mL microtube or microtiter plate
2. Add 20 µL Internal Standard solution to the sample
3. Add 40 µL MagSiMUS-TOX^{PREP} Type I Particle Mix and mix by pipetting
4. Add 250 µL premixed OPR VI (see above) and mix by 10 aspiration and dispensing cycles

This step is crucial for the performance of this kit. By the addition of precipitation reagent to the reaction mix, precipitated proteins and the bead mix form a voluminous aggregate which needs to be dispersed in a homogeneous suspension by pipetting

5. Place the samples on a magnetic separator and wait for 2 minutes until the supernatant is clear. For tubes use MM-Separator M12 + 12 P and for microtiter plates use MM-Separator 96 SBS BC
6. While avoiding contact with the pellet, transfer up to 100 µL of the supernatant to a new microtube, microtiter plate or HPLC vial for injection. Seal/close sample container to avoid evaporation and contamination

Make sure that no beads or parts of the pellet are transferred to the new sample container

5.5 Protocol for whole blood samples using methanol-based precipitation

Before use, vortex **MagSiMUS-TOX^{PREP} Type I Particle Mix** to fully resuspend the beads.

Make sure the Internal Standard solution is prepared as described in chapter 4.3.

Before use, allow products and samples to come to room temperature. Homogenize samples properly before starting the protocol.

Prepare Organic Precipitation Reagent VI (OPR VI)

- Add the content of PRZS I to the bottle of Organic Precipitation Reagent II and mix by inverting 5 times.
- Store prepared OPR VI at 2-8°C for no longer than 7 days.

Alternatively, prepare OPR VI with PRZS I and Organic Precipitation Reagent II for a specific number of samples (N):

- Add $(N+1) \times 27.5 \mu\text{L PRZS I}$ to $(N+1) \times 232.5 \mu\text{L OPR II}$

1. Transfer 25 μL blood sample to a 2 mL microtube or microtiter plate
2. Add 60 μL Lysis Buffer for whole blood and mix by pipetting. Incubate 1 minute for complete lysis of blood cells
3. Add 20 μL Internal Standard solution to the sample
4. Add 40 μL MagSiMUS-TOX^{PREP} Type I Particle Mix and mix by pipetting
5. Add 250 μL premixed OPR VI (see above) and mix by 10 aspiration and dispensing cycles

This step is crucial for the performance of this kit. By the addition of precipitation reagent to the reaction mix, precipitated proteins and the bead mix form a voluminous aggregate which needs to be dispersed in a homogeneous suspension by pipetting

6. Place the samples on a magnetic separator and wait for 2 minutes until the supernatant is clear. For tubes use MM-Separator M12 + 12 P and for microtiter plates use MM-Separator 96 SBS BC
7. While avoiding contact with the pellet, transfer up to 100 μL of the supernatant to a new microtube, microtiter plate or HPLC vial for injection. Seal/close sample container to avoid evaporation and contamination

Make sure that no beads or parts of the pellet are transferred to the new sample container

5.6 Protocol for urine samples using acetonitrile-based precipitation

Before use, vortex **MagSiMUS-TOX^{PREP} Type I Particle Mix** to fully resuspend the beads.

Make sure the Internal Standard solution is prepared as described in chapter 4.3.

Before use, allow products and samples to come to room temperature. Homogenize samples properly before starting the protocol.

1. Transfer 50 μ L urine to a 2 mL microtube or microtiter plate
2. Add 20 μ L Internal Standard solution to the sample
3. Add 20 μ L Urine Stabilization Buffer and mix by pipetting
4. Add 40 μ L MagSiMUS-TOX^{PREP} Type I Particle Mix and mix by pipetting
5. Add 250 μ L OPR I and mix by 10 aspiration and dispensing cycles

This step is crucial for the performance of this kit. By the addition of precipitation reagent to the reaction mix, precipitated proteins and the bead mix form a voluminous aggregate which needs to be dispersed in a homogeneous suspension by pipetting

6. Place the samples on a magnetic separator and wait for 2 minutes until the supernatant is clear. For tubes use MM-Separator M12 + 12 P and for microtiter plates use MM-Separator 96 SBS BC
7. While avoiding contact with the pellet, transfer up to 80 μ L of the supernatant to a new microtube, microtiter plate or HPLC vial for injection. Seal/close sample container to avoid evaporation and contamination

Make sure that no beads or parts of the pellet are transferred to the new sample container

5.7 Protocol for urine samples using methanol-based precipitation

Before use, vortex **MagSiMUS-TOX^{PREP} Type I Particle Mix** to fully resuspend the beads.

Make sure the Internal Standard solution is prepared as described in chapter 4.3.

Before use, allow products and samples to come to room temperature. Homogenize samples properly before starting the protocol.

Prepare Organic Precipitation Reagent VI (OPR VI)

- Add the content of PRZS I to the bottle of Organic Precipitation Reagent II and mix by inverting 5 times.
- Store prepared OPR VI at 2-8°C for no longer than 7 days.

Alternatively, prepare OPR VI with PRZS I and Organic Precipitation Reagent II for a specific number of samples (N):

- Add (N+1)***27.5 µL PRZS I** to (N+1)***232.5 µL OPR II**

1. Transfer 50 µL urine to a 2 mL microtube or microtiter plate
2. Add 20 µL Internal Standard solution to the sample
3. Add 20 µL Urine Stabilization Buffer and mix by pipetting
4. Add 40 µL MagSiMUS-TOX^{PREP} Type I Particle Mix and mix by pipetting
5. Add 250 µL premixed OPR VI (see above) and mix by 10 aspiration and dispensing cycles

This step is crucial for the performance of this kit. By the addition of precipitation reagent to the reaction mix, precipitated proteins and the bead mix form a voluminous aggregate which needs to be dispersed in a homogeneous suspension by pipetting

6. Place the samples on a magnetic separator and wait for 2 minutes until the supernatant is clear. For tubes use MM-Separator M12 + 12 P and for microtiter plates use MM-Separator 96 SBS BC
7. While avoiding contact with the pellet, transfer up to 100 µL of the supernatant to a new microtube, microtiter plate or HPLC vial for injection. Seal/close sample container to avoid evaporation and contamination

Make sure that no beads or parts of the pellet are transferred to the new sample container

6. Troubleshooting

Problem	Probable cause	Suggestion
Pellet formation not sufficient	Magnetic separator not suitable	Use suggested separators: 1.5 and 2 mL tubes: MM-Separator M12 + 12 P microtiter plate: MM-Separator 96 SBS BC
	Not enough beads in the sample mix	Before use vortex MagSiMUS-TOX ^{PREP} Type I Particle Mix into a homogeneous suspension
	Insufficient mixing of sample with beads and precipitation agent	Make sure to mix until a homogeneous suspension is formed
Inconsistent signal intensity detected (peak area)	Evaporation of organic solvents during or after sample preparation	Keep containers closed when possible
Bead present in purified sample	Carry-over of beads during final sample transfer step	Be careful not to disturb the bead pellet. If possible, use smaller transfer volume for easier pipetting
Peak broadening or tailing of LC-MS/MS chromatogram	LC buffer conditions do not match the precipitation agent used in the protocol	Check the compatibility of the precipitation agent with your LC mobile phase. For mobile phase containing methanol use protocol 5.4, 5.5 or 5.7, for acetonitrile use protocol 5.3 or 5.6

7. Automation

MagSiMUS-TOX^{PREP} Type I is suitable for use in automated sample preparation process. Since no heating, cooling, centrifugation or vortexing is required, the protocols are easily performed on most automated liquid handling workstations without the need of installing additional modules.

For details how to automate the protocols on specific liquid handling workstations, please look at the automation section on our website: www.magtivio.com or e-mail to support@magtivio.com.

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