

## METHOD AT A GLANCE

## FLOMASS<sup>®</sup> p-CRESYL SULFATE AND INDOXIL SULFATE (TOTAL AND FREE) IN SERUM

p-Crsyl Sulfate (pCS) and Indoxyl Sulfate (IS) are uremic toxins synthesized by gut bacteria and predominantly linked to albumin.

Since only free uremic toxins are able to cross the dialytic membranes, their removal is limited in the dialysis therapies currently in use.

Increase of pCS and Indoxyl Sulfate can lead to the formation of a reversible bond to albumin in blood and, mainly in dialysis patients, can cause renal inflammation and consequent risk of complications, such as cardiovascular diseases.



### HPLC-MS/MS system conditions

Ionization: ESI negative mode MS/MS: specific MRM Injection volume: 2 μL (variable according to instrumental sensitivity) Running time: 6 min Column heater: 40°C Column conditioning: column should be conditioned for 5 min at chromatographic

gradient initial condition. Then run 3 blank injections (MPA only) using the gradient as indicated in IFU



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### Sample preparation

#### TOTAL fraction

- Pipette 50 µL of serum in 1.5 o 2 ml PP vial
- Prepare Mix-T solution: Internal Standard Mix to Solution Precipitant at ratio 1:10. Vortex
- Add to each sample 100 µL of Mix-T solution. Vortex 30 sec
- Centrifuge 5 min at 10000 rpm
- Transfer 100 µL supernatant in a new 1.5- or 2.0-ml PP vial\*
- Add 900 µL of H<sub>2</sub>O. Vortex.
- Transfer 100 µL in autosampler vial with plastic adapter and analyze with HPLC-MS/MS technique

#### \* Highly sensitive instruments need further dilution

#### FREE fraction

- Pipette 300 µL of serum in vials with 10 kDa filter
- Centrifuge 7 min at 10000 rpm
- Transfer 50 µL of ultra-filtrate in a new 1.5- o 2-ml PP vial
- Prepare Mix-L solution: Internal Standard Mix to Solution Precipitant at ratio 1:100. Vortex
- Add to each sampl100 µL Mix-L solution. Vortex for 30 sec
- Centrifuge for 5 min at 10000 rpm
- Transfer 100 µL in autosampler vial with plastic adapter and analyze with HPLC-MS/MS technique\*

## Performance

ANALYTE	LINEARITY (ng/mL)	LLOD (ng/mL)	LLOQ (ng/mL)	CV% INTRA	CV% INTER
p-Cresyl Sulfate	0.2 - 450	0.06	0.20	1.76 – 5.82	2.34 - 4.75
Indoxyl Sulfate	0.31 - 500	0.09	0.31	4.14 - 12.8	3.4 - 6.9

## Ordering guide

EUM03200	FloMass® p-Cresyl Sulfate and Indoxyl Sulfate (total and free) in Serum	200 assays
EUM03041	6-Levels Calibrators, lyophil.	2 x 6 x 0.5 mL
EUM03051	2-Levels Controls, lyophil.	2 x 2 x 0.5 mL
EUM00C03	Chromatographic Column	1 pc

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