

Instruction sheet

AFFINIMIP® SPE Estrogens cartridges

CLEAN-UP PROCEDURE OF ESTROGENS FROM WATER or PLASMA

Users should read all instructions before using this kit.

For research use only (RUO)

AFFINIMIP® SPE ESTROGENS is:

- Developed and manufactured by AFFINISEP

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Method for Selective Phase Extraction of Estrogens using Molecularly Imprinted Polymers

1 PRODUCT INFORMATION

AFFINIMIP® SPE Estrogens has been developed to selectively extract Estrogens compounds from complex matrices such as plasma and water.

By using AFFINIMIP® SPE, the expected result is a clean-up and a pre-concentration of the sample at trace levels.

2 PRINCIPLE OF AFFINIMIP® SPE

AFFINIMIP® SPE is a solid phase obtained by a polymerisation process to create a threedimensional network that recognizes the shape and functional group positions of a template molecule. The AFFINIMIP® SPE selectivity comes from the technology of molecularly imprinted polymer (MIP) used during the synthesis.

3 PRODUCT INFORMATION

Description of the kit

Each solid phase extraction (SPE) cartridge **AFFINIMIP®** SPE Estrogens contains 100mg of sorbent in a 3mL cartridge.

Other cartridges format can be supplied for high or small loading volumes such as 1mL, 6mL, 15mL, 20mL cartridges.

Information and storage

Storage: Room temperature. Each cartridge has a single use.

4 PRECAUTIONS FOR USE

SPE methods developed for C18 or other sorbents are not appropriated for AFFINIMIP® SPE Estrogens. The extraction procedure described below has been optimized for the extraction of Estrogens from water and plasma samples. For the treatment of another matrix, please contact us to adapt the extraction procedure.



5 GENERAL INSTRUCTIONS FOR SPE

5.1 Equipments required

In addition to standard laboratory equipments, the following material is required for the use of AFFINIMIP® SPE cartridges:

- SPE vacuum manifold or a SPE automate
- Nitrogen Mini-vap evaporator or a vacuum concentrator to dry the collected sample

5.2 <u>Flow rate</u>

It is very important to follow the flow rate given in the protocol.

Most especially for the loading, if the sample flow rate is too high, components may not interact sufficiently with the sorbent and the Estrogens recovery yield will be lower.

5.3 <u>Contamination of ultrapure water</u>

Tap water is often contaminated by the presence of Endocrine Disrupting Chemicals (EDC) such as Estradiol. These products can also be present in **ultrapure water** if the purification cartridges used are not adapted to remove them.

Please check that the ultrapure water used for the equilibration and washing is not contaminated.

5.4 <u>Preparation process</u>

For the MIP preparation, a template is required. Estrogens analogues were used instead of Estrogens to prevent a false positive signal in case of bleeding.

5.5 <u>Preparation of solution</u>

- Solution 60/40 ultrapure water / Acetonitrile (v/v)

In a 10mL-volumetric flask, add 4mL of ACN and complete with ultrapure water.

5.6 General information on the loading solution



Users developing their own extraction method must take into considerations that the composition of the Organic solvents – Water loading solution must contains a maximum of 25% Organic solvents.

6 CLEAN-UP PROCEDURE OF ESTROGENS FROM WATER:

Step (Flow rate)	AFFINIMIP® SPE Estrogens (100mg/3mL)		
Equilibration with (2 drops/s)	 3mL Acetonitrile 3mL ultrapure water Do not allow the cartridge to dry during conditioning 		
Loading (L) (1 drop every 2 seconds)	 Up to 50mL water (to be analyzed) 		
Washing of interferents	3mL ultrapure water		
(1 drop/s)	• 3mL of 60/40 ultrapure water / Acetonitrile		
Drying	 Apply vacuum or nitrogen flow through cartridge during 3-5 min This step is only necessary if you evaporate the elution solution 		
Elution (E)	3mL Methanol		
(1 drop/s)			

The elution fraction (E) is evaporated until dryness under nitrogen with a mini-vap evaporator at room temperature (or a centrifugal evaporator). The residue is dissolved in 0.5mL of mobile phase for further analysis. Alternatively, the elution may be diluted to a known volume by addition of water for further analysis.



7 CLEAN-UP PROCEDURE OF ESTROGENS FROM PLASMA:

7.1 <u>Preparation of the loading solution</u>

In a 20mL-volumetric flask: add 4mL of plasma and completed with 16mL of ultrapure water

7.2 Protocol for the clean-up of Estrogens from plasma

Step (Flow rate)	AFFINIMIP® SPE Estrogens (100mg/3mL)		
Equilibration with (2 drops/s)	 3mL Acetonitrile 3mL ultrapure water Do not allow the cartridge to dry during conditioning 		
Loading (L) (1 drop every 2 seconds)	 Up to 9mL of the loading solution 		
Washing of interferents	3mL ultrapure water		
(1 drop/s)	 3mL of (60/40) ultrapure water/ Acetonitrile (v/v) 		
Drying	 Apply vacuum or nitrogen flow through cartridge during 3-5 min This step is only necessary if you evaporate the elution solution 		
Elution (E) (1 drop/s)	• 3mL Methanol		

The elution fraction (E) is evaporated until dryness under nitrogen with a mini-vap evaporator at room temperature (or a centrifugal evaporator). The residue is dissolved in 0.5mL of mobile phase for further analysis. Alternatively, the elution may be diluted to a known volume by addition of water for further analysis.



PRODUCTS LIST

AFFINIMIP [®] SPE Products	Designation	Description
Multimyco10	AFFINIMIP [®] SPE Multimyco10	selective SPE cartridges 3mL for ZON, OTA, HT-2, T-2, Aflatoxins and Fumonisins
Zearalenone	AFFINIMIP [®] SPE Zearalenone	selective SPE cartridges 3mL for ZON
Ochratoxin A	AFFINIMIP [®] SPE Ochratoxin A	selective SPE cartridges 3mL for OTA
	AFFINIMIP [®] SPE Patulin	selective SPE cartridges for Patulin
Patulin	AFFINIMIP [®] SPE Patulin & Pectinase kit	kit of selective SPE cartridges for Patulin + 50mL pectinase enzyme solution
Deoxynivalenol	AFFINIMIP [®] SPE Deoxynivalenol	selective SPE cartridges 6mL for DON
Phenolics	AFFINIMIP [®] SPE Phenolics	selective SPE cartridges for Phenolic compounds
Estrogens	AFFINIMIP [®] SPE Estrogens	selective SPE cartridges for Estrogens
Zeranol Residues	AFFINIMIP [®] SPE Zeranol Residues	selective SPE cartridges for Zeranol Residues
Bisphenol A	AFFINIMIP [®] SPE Bisphenol A	selective (PP or Glass) SPE cartridges for Bisphenol A
FumoZON	AFFINIMIP [®] SPE FumoZON	selective SPE cartridges for Fumonisins and Zearalenone
Chloramphenicol	AFFINIMIP [®] SPE Chloramphenicol	selective SPE cartridges for Chloramphenicol
Tamoxifen	AFFINIMIP [®] SPE Tamoxifen	selective SPE cartridges for Tamoxifen
Catecholamines	AFFINIMIP [®] SPE Catecholamines	selective SPE cartridges for Catecholamines
Catecholamines	AFFINIMIP [®] SPE Catecholamines	selective SPE cartridges for Catecholamines
Metanephrines	AFFINIMIP [®] SPE Metanephrines	selective SPE cartridges for Metanephrines
Amphetamines	AFFINIMIP [®] SPE Amphetamines	selective SPE cartridges for Amphetamines
PECTINASE	Pectinase solution	50 mL pectinase enzyme solution
AttractSPE [™] Products	Designation	Description
w/o	AttractSPE [™] W/O	HLB SPE cartridges sorbent
SCX	AttractSPE [™] SCX	Strong Cation Exchange SPE cartridges sorbent
wcx	AttractSPE [™] WCX	Weak Cation Exchange SPE cartridges sorbent
SAX	AttractSPE [™] SAX	Strong Anion Exchange SPE cartridges sorbent
WAX	AttractSPE [™] WAX	Weak Anion Exchange SPE cartridges sorbent
DVB	AttractSPE™ DVB	Reversed Phase Copolymer SPE cartridges sorbent
Anionic & Cationic AttractSPE polymeric cartridges	AttractSPE™ KIT	Kit of 10 cartridges of each sorbent (SAX, WAX, WCX, SCX)

For more information:

For more information on our products & services, please visit <u>www.polyintell.com</u>