

MYCOPHENOLIC ACID IN PLASMA/SERUM BY UV – FAST – CODE Z01960

BIOCHEMISTRY

Mycophenolic Acid or Mycophenolate is an immunosuppressant drug used to prevent rejection in organ transplantation. It was initially marketed as the prodrug mycophenolate mofetil to improve oral bioavailability. Mycophenolate is derived from the fungus *Penicillium stoloniferum*. Mycophenolate mofetil is metabolised in the liver to active moiety mycophenolic acid. It inhibits **Inosine monophosphate dehydrogenase**, the enzyme which controls the rate of synthesis of guanine monophosphate in the *de novo* pathway of purine synthesis used in the proliferation of B and T lymphocytes. Mycophenolate is potent and can be used in place of the older anti-proliferative azathioprine. It is usually used as part of triple-therapy including a calcineurin inhibitor (cyclosporin or tacrolimus) and prednisolone.



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This product fulfills all the requirements of Directive 98/79/EC on in vitro diagnostic medical devices (IVD). The declaration of conformity is available upon request.

N° 002	Mycophenolic acid in plasma/serum by UV	09/2024
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TECHNICAL FEATURES

Principle of the Method :

Mycophenolic Acid are separated treating the serum/plasma with a suitable precipitant and they are directly injected into HPLC.

Recovery :

> 98 %

Sensitivity (LLOD) :

0,04 mg/l

Minimum Concentration analyzable (LLOQ) :

0,1 mg/l

Linearity :

0,1 – 60 mg/l

Reference value :

4 - 15 mg/l

Accuracy intra serie (relative error %) :

Ci	Cs
2 mg/l	10 mg/l
11,5%	5,8%

Accuracy inter serie (relative error %) :

Ci	Cs
2 mg/l	10 mg/l
14,9%	5,3%

Reproducibility intra serie (coefficient of variation %) :

C LLOQ	Cm	CA
0,1 mg/l	5 mg/l	20 mg/l
5,0%	3,0%	2,0%

Reproducibility inter serie (coefficient of variation %) :

C LLOQ	Cm	CA
0,1 mg/l	5 mg/l	20 mg/l
13,7%	5,6%	3,0%

Coefficient of correlation R2 + Dev. std:

0,9933 ± 0,0025

Components of the kit :

All the components are ready-to-use. Stability: 3 years at room temperature ($\leq 20^{\circ}\text{C}$). The Calibrator is stable 48 months at 2–8 °C.

Reagent A – Deproteinization Sol. with Internal Standard, **1 x 10 ml**

Reagent B – Diluting Solution, **1 x 15 ml**

Reagent C – Test Solution, **1 x 0,5 ml**

Store at 2-8 °C

Plasma Calibrator lyophil., **1 x 2 ml**

Code Z01916 (packed separately – see data sheets)

Reagent M – Mobile Phase, **3 x 500 ml**

Minimum Instrumental equipment required:

Isocratic HPLC System with loop of 20 μ l
Spectrophotometric Detector $\lambda = 210 \text{ nm}$
Chromatograms Recorder

Optional Equipment:

Autosampler
Operational Computer

Plasma Collect Procedure :

Collect 3 ml of whole blood in a suitable test tube without gel and with Heparin as anticoagulant. Centrifuge at 4000 rpm for 5 minutes. Separate the serum and store at -20°C . Stable 4 weeks.

PREANALYTICAL PROCEDURE

Pipette in a tube :

- 450 µl of H₂O HPLC grade
- 50 µl of **Reagent C – Test Solution**

INJECTION :

- Inject 20 µl of this solution in the chromatographic system.

Verify that the Test Solution has retention time similar to fig. 1. If the Test is all right you can start with the analytical procedure; if not, check the functionality of the analytical system.

Important : Don't use this solution to calibrate!

ANALYTICAL PROCEDURE

STEP 1 : Sample Preparation

Pipette in a tube:

	Calibrator	Controls	Samples
Calibrator	100 µl		
Controls		100 µl	
Samples			100 µl
Reagent A – Deprot. with Internal Standard	100 µl	100 µl	100 µl

Warning: deproteinize directly on Vortex for at least 10 sec.

STEP 2 : Centrifuge at 14.000 rpm for 10 minutes.

STEP 3 : Diluting

Pipette in a tube 150 µl of clear supernatant and add 150 µl of **Reagent B – Diluting Solution**

Vortex for 10 sec.

N.B.: at this step, the eluate is stable 2 days at 2-8 °C

INJECTION :

- Inject 20 µl of this solution in the chromatographic system.

MYCOPHENOLIC ACID BY UV - Warnings

REAGENT C : TEST SOLUTION

Mycophenolic Acid	about 100 mg /l
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DETECTOR UV/VIS PARAMETERS

λ	210 nm
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HPLC COLUMN PROTECTION

To save the analytical column Reverse Phase POROSHELL EC C18 (50 x 4,6 mm – 2,7 μ m), the use of Javelin Prefilters (1 x 10 pcs), code S90199511 is obligatory.

HPLC COLUMN CONDITIONING

Install a <u>new</u> analytical column Reverse Phase POROSHELL EC C18 (50 x 4,6 mm – 2,7 μ m). Disconnect the detector and flux 30 ml of a solution of H₂O : Methanol (20 : 80 v/v) set flow at 1,0 ml / min. Don't recycle the washing solutions. Flux 30 ml of a solution of H₂O : Methanol (80 : 20 v/v) set flow at 1,0 ml / min. Condition the column with the mobile phase at a flow of 1ml / min. discharge first 20 ml. Finally inject the derivatized Chemical Standard and verify the quality of the HPLC run. It is possible to make analysis at recycling phase. If room temperature is > 20 °C store the Mobile Phase at 2-8 °C between an analytical session and another.

COLUMN CLEANING

Disconnect the detector. Flush 15 ml of H₂O: Methanol or Acetonitrile (80: 20 v/v) and set flow at 1,0 ml / min and discharge. Flush a solution of H₂O: Methanol or Acetonitrile (30: 70 v/v) for 30 minutes for download. It is recommended to wash the analytical column at the end of each session. The column is stored in a solution of H₂O : Acetonitrile or Methanol (30 : 70 v / v)

INJECTION NEEDLE WASH

Wash in a solution of H ₂ O / Ethanol or Methanol or Acetonitrile (80 : 20 v/v).

HPLC PARAMETERS

LOOP	20 μ l
FLOW	1,0 ml/min.
PRESSURE	About 100 bar

ACCESSORIES AND CONSUMABLES

CODE	DESCRIPTION	PACKAGING
Z01916	Calibrator for Mycophenolic Acid in serum and/or in plasma	4 x 2 ml
Z01919	Control per for Mycophenolic Acid in serum and/or in plasma – Levels 1 and 2	2 x 5 x 2 ml
Z699975902	Poroshell 120, EC-C18 (50 x 4,6 mm - 2,7 μ m) Analytical Column	1 Pc
S90199511	Javelin Colfilters	1 x 10 Pcs
S51843550	Clear glass vials with reduced volume from 1,5 ml to 15 μ l	1 x 100 Pcs
S51820717	Caps for Clear glass vials with reduced volume from 1,5 ml to 15 μ l	1 x 100 Pcs

MYCOPHENOLIC ACID IN PLASMA/SERUM BY UV
(Reference Chromatograms)

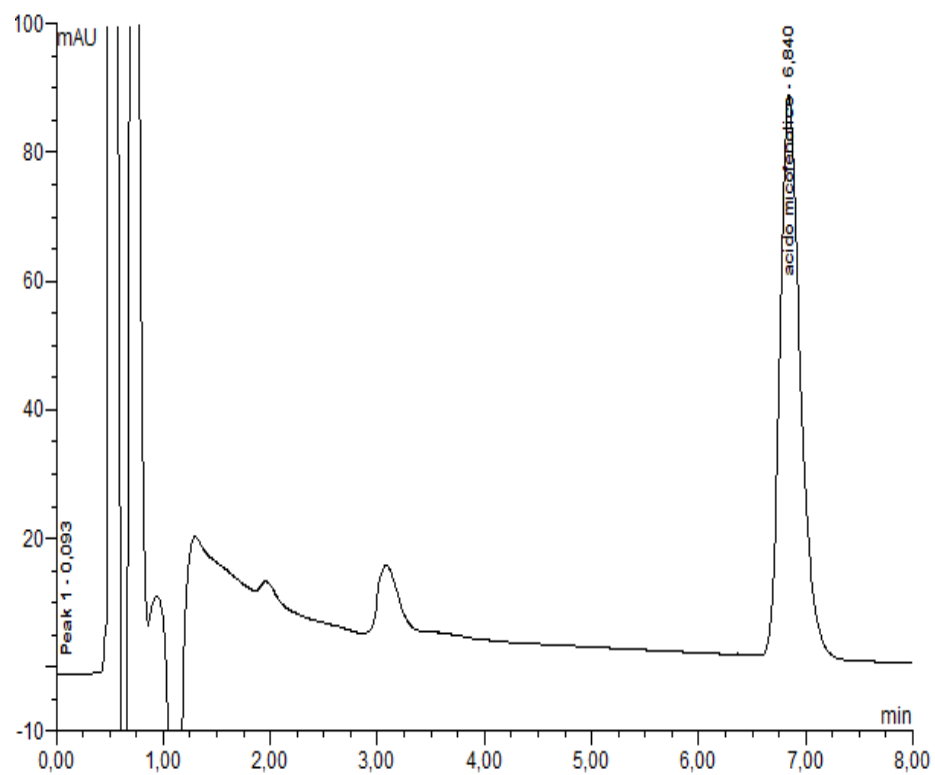


Fig. 1	Test Solution
	R.T. 6.8 Mycophenolic Acid

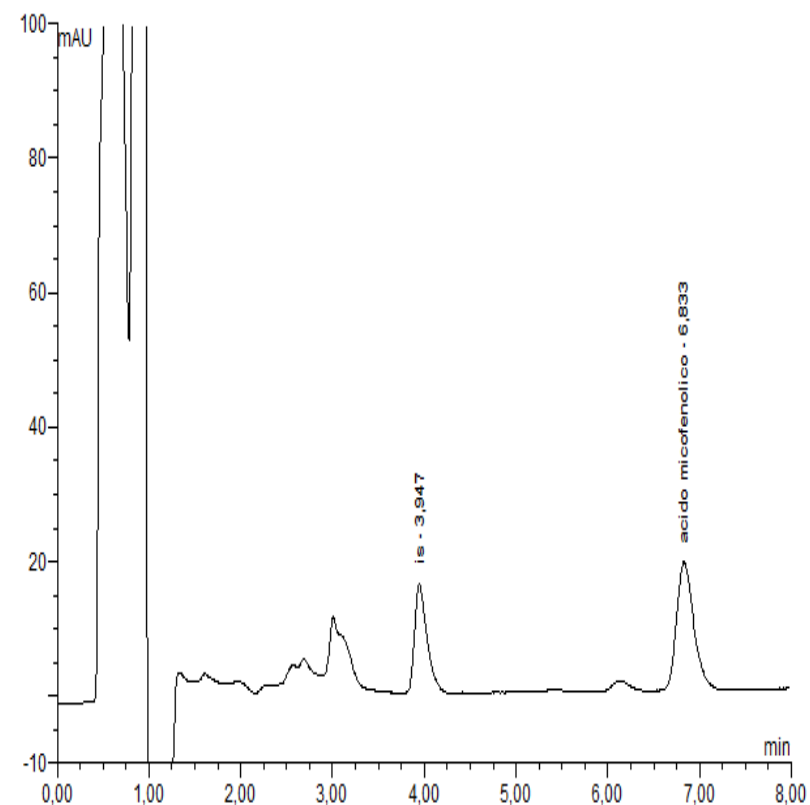


Fig. 2	Plasma Calibrator
	R.T. 3.9 Internal Standard
	R.T. 6.8 Mycophenolic Acid