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Mycophenolic Acid ELISA Kit

(Catalog # E4819-100; 96 assays, Store kit at -20°C)

I. Introduction:

Mycophenolic acid (MPA), also known as mycophenolate, is produced by a mold species called *Penicillium brevicompactum* and is an immunosuppressive agent used to prevent rejection following kidney, heart, and liver transplants. The mechanism of action of MPA is to directly bind to inosine-5′-monophosphate dehydrogenase (IMPDH) which is an enzyme essential for the de novo synthesis of guanosine-5'-monophosphate from inosine-5'-monophosphate. MPA is a potent, reversible, non-competitive inhibitor of IMPDH and it can specifically inhibit proliferative responses of T- and B-lymphocytes by blocking the de novo pathway of guanosine nucleotide synthesis. MPA can be also used in treating different autoimmune diseases and similar immune-mediated disorders including Crohn's disease, Behçet's disease, pemphigus vulgaris, small vessel vasculitides, rheumatoid arthritis and psoriasis. There are some common side effects such as nausea, diarrhea, anemia, gastrointestinal bleeding and infections and therefore it is important to monitor MPA levels in the body. The traditional techniques/instruments (HPLC or GC-MS) for detecting MPA are expensive, laborious, and time-consuming. On the other hand, immunoassay techniques, such as ELISA, are commonly preferred as simple, reliable and rapid methods. BioVision's MPA ELISA kit is a competitive-based ELISA that can detect MPA in different biological samples such as serum and urine. It can detect MPA (1 – 256 ng/ml) within 90 minutes (LOD 0.5 ng/ml).

II. Applications:

In vitro quantitative determination of MPA

III. Sample Type:

Serum and urine samples

IV. Kit Contents:

Components	E4819-100	Cap Code	Part Number
ELISA Microplate	8 X 12 Strips	--	E4819-100-1
MPA Standard	2 vials	Yellow	E4819-100-2
HRP Conjugate Stock	12 µl	Blue	E4819-100-3
Antibody	7 ml	NM/Red	E4819-100-4
TMB substrate	12 ml	Amber	E4819-100-5
Stop Solution	10 ml	NM/Blue	E4819-100-6
Wash Buffer (10X)	50 ml	NM	E4819-100-7
Sample Diluent	20 ml	NM	E4819-100-8
Serum Solution	1 ml	Brown	E4819-100-9
Standard Buffer	25 ml	WM	E4819-100-10
Conjugate Buffer	7.5 ml	NM/Green	E4819-100-11
Plate Sealers	4	--	E4819-100-12

V. User Supplied Reagents and Equipment:

- Microplate reader capable of measuring absorbance at 450 and 650 nm
- Precision pipettes with disposable tips
- Clean eppendorf tubes for preparing standards and sample dilutions

VI. Storage and Handling:

The entire kit may be stored at -20°C for up to 12 months from the date of shipment. Opened kit is stable for 2 months at 4°C.

VII. Reagent and Standard Preparation:

Bring all reagents to room temperature before use. Before using the kit, spin tubes and bring down all components to the bottom of tubes.

- **Antibody, TMB Substrate, Stop Solution, Sample Diluent, Serum Solution, Standard Buffer and Conjugate Buffer:** Ready to be used. After use, store them at 4°C.
- **HRP Conjugate Stock**: Spin briefly before opening the tube. Pipet 12 µl of HRP Conjugate Stock into Conjugate Buffer (7.5 ml) bottle to prepare **conjugate working solution**. Vortex the bottle for a minute. The conjugate working solution is stable at 4°C for 2 months.
- **Wash Buffer (10X)**: Bring bottle to room temperature. If crystals are present, warm up to room temperature and mix gently until the crystals are completely dissolved. Prepare 100 ml of 1X Wash Buffer by diluting 10 ml of Wash Buffer (10X) with 90 ml deionized water. The 1X solution can be stored at 4°C for one month.
- **MPA Standards**: Reconstitute the MPA standard by adding 1.5 ml of Standard Buffer to prepare 256 ng/ml standard (S6). Allow solution to sit at room temperature for 10 minutes, then gently vortex to mix completely. Dilute S6 two-fold (e.g. 0.25 ml with 0.25 ml of Standard Buffer) to prepare S5. Perform fourfold serial dilutions starting from S6 (e.g. mix 0.25 ml standard with 0.75 ml of Standard Buffer) to prepare standards S4 to S1 sequentially. S0 is Standard Buffer. The standards are stable at -20°C for up to 3 weeks.

Standards	S0	S1	S2	S3	S4	S5	S6
Concentrations (ng/ml)	0	1	4	16	64	128	256

VIII. Sample Preparation:

- **Urine**

1. Centrifuge 0.5 ml of urine at 10,000 x g for 5 min to remove any precipitate and collect the supernatant.
2. Dilute the supernatant 10-fold with Sample Diluent (For example, mix 50 µl of urine with 450 µl of Sample Diluent.)
3. Use 50 µl per well for the assay.

- **Serum**

1. Add 10 µl of Serum Solution into 190 µl of serum in an Eppendorf tube and vortex well. Incubate samples at 37°C for 45 min.
2. After the first incubation, incubate samples at 85-90°C for 10 min.
3. After 10 min, dilute the serum sample tenfold using the Sample Diluent (For example, mix 20 µl of serum with 180 µl of Sample Diluent.).
4. Use 50 µl per well for the assay.

IX. MPA ELISA Assay Protocol:

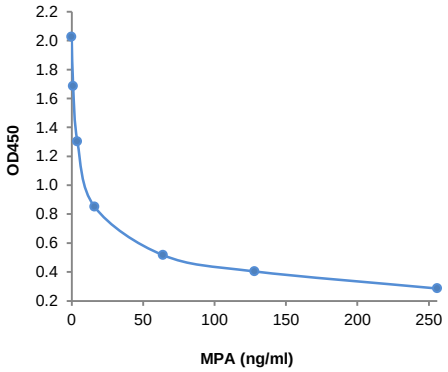
Notes: We recommended that all standards and samples are run in duplicate. A Standard curve must be run each time an assay is performed.

1. Prepare all reagents, standards and samples as sections VII and VIII specify respectively.
2. Add 50 µl of **Standards or Samples** per well. Then add 50 µl of **conjugate working solution** and 50 µl of **Antibody** to the above wells.
3. Cover the microtiter plate with plate sealer and mix well. Incubate the plate at room temperature (25°C) for 60 min.
4. Aspirate all reagents and wash each well 5 times: add 250 µl of **1X Wash Buffer** and incubate for 30 seconds. Remove 1X Wash buffer completely before the next wash. (Complete removal of wash buffer is essential for accurate results.) Repeat wash step 4 more times.
5. Add 100 µl of **TMB Substrate** to each well. Tap or shake the plate occasionally to ensure complete mixing.
6. Check the OD at 650 nm for the well containing no MPA (S0). When its reading is approximately 0.8, add 50 µl of **Stop Solution** and gently tap the plate to ensure thorough mixing.
7. Measure the OD at 450 nm.

X. Calculation:

The Standard Curve is done by plotting the relative absorbance of the standards vs. MPA concentrations. The concentration of MPA of each sample, which can be read from the calibration curve, is multiplied by the corresponding dilution factor.

A.



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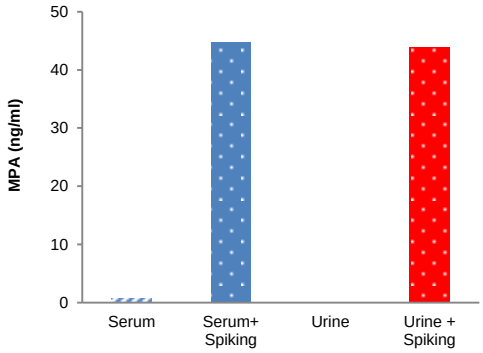

Figures. **A.** MPA standard curve (*This standard curve is for demonstration only. A standard curve must be run with each assay*). **B.** Spike recovery experiment: Human serum and urine samples were spiked with MPA (50 ng/ml) and showed > 85% recovery.

XI. RELATED PRODUCTS:

Gentamicin (serum/urine) ELISA Kit (Cat. No. K4315)
Folic Acid ELISA Kit (Cat. No. E4523)
Caffeine Acid ELISA Kit (Cat. No. E4558)
His-Tag Protein ELISA Kit (Cat. No. E4550)
DYKDDDDK-Tag Protein ELISA Kit (Cat. No.E4700)
Isoniazid ELISA Kit (Cat. No. E4765)
Ampicillin ELISA Kit (Cat. No. E4350)
Quinolone ELISA Kit (Cat. No. E4530)
Vancomycin ELISA Kit (Cat. No. E4605)
GST Tag ELISA Kit (Cat. No. E4690)
Bisphenol A ELISA Kit (Cat. No. E4722)
Carnosine ELISA Kit (Cat. No. E4766)

FOR RESEARCH USE ONLY! Not to be used on humans.