



# 1-MeTIQ ELISA

Procedure

EK-MTQ

A Commitment to Diagnostics

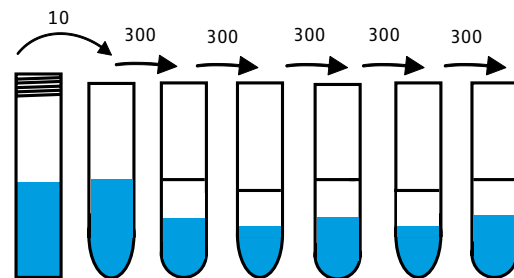
## Pre-Analytics

Sample specimen and volume: 1 ml serum, 1 g brain and at least 50 µl CSF

Sample storage: at 2-8 °C up to 7 days at -20 °C for longer storage

## Preparation of Standard Curve

Reconstitute calibrator. Prepare serial dilutions of calibrator as follows: Pipet 990 µl of Incubation buffer (IB) into tube S1, 700 µl IB into tubes S2, S4 and S6, and 600 µl IB into tubes S3 and S5. Pipet 10 µl of Calibrator (1 µg/ml) into tube S1 and vortex. Transfer 300 µl from S1 to S2, vortex. Transfer 300 µl from S2 to S3, vortex and continue until the series is completed.



Blank	S1	S2	S3	S4	S5	S6
1	10	3	1	0.3	0.1	0.03
µg/ml	ng/ml	ng/ml	ng/ml	ng/ml	ng/ml	ng/ml

## Sample Preparation

### Brain Tissue

Homogenization:

1. Add 10 x (v/w) 0.4 M perchloric acid to the tissue (e.g. 1 g tissue + 10 ml acid). Homogenize for a few seconds with an ultra-turrax dispenser followed by 10 minutes treatment in an ultrasonic bath.
2. Centrifuge at 4000 x g at 2 - 8 °C for 20 min. Collect supernatant.
3. Repeat the homogenization (steps 1 and 2) with 10 x 0.4 M perchloric acid.
4. Combine both supernatants and perform the solid phase extraction described below.

Extraction: refer to procedure described for serum

### CSF

Sample dilution 1:2 in Incubation buffer (1+1)

### Serum

Perform Solid Phase Extraction with Strata™ columns following steps 1-8. Extraction under vacuum is required.

1. Conditioning: 2 ml methanol
2. Equilibration: 2 ml deionized H2O
3. Load Sample: 1 ml serum/homogenate
4. Wash: 2 ml 5% methanol
5. Dry: 1 minute
6. Elute Analyte: 2 ml methanol, use a fresh tube

Do not exceed a flow rate of 1-2 ml per minute.

## ELISA Procedure

Precoated microtiter plate

↓ ↻ wash 2x

50 µl calibrators , controls and samples

add 50 µl of Biotin conjugate

add 50 µl of 1-MeTIQ-antiserum

120' ↓ ↻ incubate 2 h at 18-25°C  
↓ ↻ wash 3 x

add 100 µl of Enzyme label

60' ↓ ↻ incubate 60 minutes at 18-25°C  
↓ ↻ wash 3 x

add 100 µl of TMB substrate

30' ↓ ↻ incubate 30 min at 18-25°C in the dark

add 100 µl of Stop solution

→ Read at 450 nm

Time to Result: 210 minutes



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Characteristics

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## Intended Use

The BÜHLMANN 1-MeTIQ ELISA kit is designed for the quantitative *in vitro* diagnostic determination of 1-Methyl-1,2,3,4-Tetrahydroisoquinoline (1-MeTIQ) in serum, cerebrospinal fluid and brain tissue.

## Principle of the Assay

1-MeTIQ present in the samples competes with biotinylated 1-MeTIQ for the binding sites of the specific rabbit anti-1-MeTIQ antibody. Simultaneously, the complexes bind to the anti-rabbit-antibody coated to the microtiter plate. Horseradish peroxidase (HRP) added during a second incubation step binds to the Biotin-Ab complexes. Tetramethylbenzidin (TMB) Substrate is formed into a coloured product inverse proportional to the amount of 1-MeTIQ present in the sample.

## Test Components

Reagents	EK-MTQ	Comments
Microtiter Plate	12 x 8 wells	
Incubation buffer	1 x 100ml	ready to use
Calibrator 1 µg/ml	1 ml	ready to use
Control Low/High	2 x lyoph.	add 1 ml Inc. Buffer
Antiserum	1 x 5.5 ml	ready to use
Biotin Conjugate	1 x 5.5 ml	ready to use
Wash Buffer	1 x 100 ml	Dilute with 900 ml H <sub>2</sub> O
Enzyme Label	1 x 11 ml	ready to use
TMB Substrate	1 x 11 ml	ready to use
Stop Solution	1 x 11 ml	ready to use

## Assay Performance Data

**Effective Range** 0.100 -10 ng/ml

**Analytical Sensitivity** 0.015 ng/ml

**Functional Sensitivity** 0.1 ng/ml

**Precision**

**Intra-Assay Precision (serum)** 8.7 %

n=4 range: 0.132-6.670 ng/ml  
20 duplicates; CV range: 6.8 - 13.7 %

**Inter-Assay Precision** 16.0 %

n=3; range: 0.07-6.87 ng/ml  
18-20 repetitions; CV range: 6.7 - 17.0 %

**Dilution Linearity**

**Serum:** n = 2; diluted 1:1.5 to 1:38  
recovery range: 70 - 116%

**CSF:** n = 2; diluted 1:2 to 1:64  
recovery range: 90 - 120%

**Spiking-Recovery**

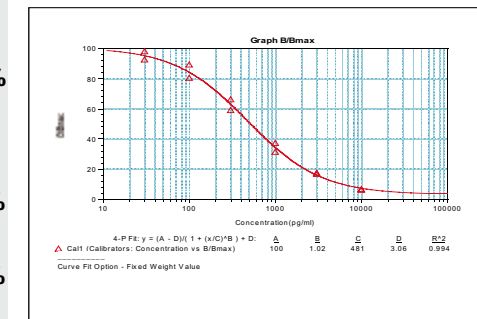
**Serum:** n=2; spiked with 0.3-8.1 ng/ml  
recovery range: 77-119 %

**CSF:** n=2; spiked with 0.3-8.1 ng/ml  
recovery range: 87-127 %

## Cross Reactivity (at 50 % B/B0)

Substance	Cross Reactivity
1,2,3,4-tetrahydroisoquinoline (TIQ)	1.6 %
3-methyl-1,2,3,4-tetrahydroisoquinoline	< 1 %
1,2,3,4-tetrahydroquinoline (TQ)	< 1 %
1-methylisoquinoline (1-MeIQ)	< 1 %
(+/-)-Salsolinol HCl	< 1 %
6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline HCl	< 1 %
6,7-dimethoxy-1-methyl-1,2,3,4-tetrahydroisoquinoline HCl	< 1 %
7-dimethoxy-3-methyl-1,2,3,4-tetrahydroisoquinoline HCl	< 1 %

## Typical Standard Curve



Product Code:

EK-MTQ

96 wells



BÜHLMANN Laboratories AG  
Baselstrasse 55  
CH-4124 Schönenbuch/Basel  
Switzerland

Phone +41 61 487 12 12  
Fax orders +41 61 487 12 99  
info@buhmannlabs.ch  
www.buhmannlabs.ch