1-MeTIQ

1-Methyl-1,2,3,4-Tetrahydroisoquinoline ELISA

EK-MTQ 96 tests

For Research Use Only (RUO)

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INTENDED USE

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

PRINCIPLE OF THE ASSAY

The BÜHLMANN 1-MeTIQ ELISA is a competitive immunoassay. A polyclonal antibody (Ab) specific for rabbit antibodies has been coated onto the microtiter plate wells. During the first incubation, 1-MeTIQ present in the samples competes with biotinylated 1-MeTIQ for rabbit anti-1-MeTIQ-antibody specific binding sites. Simultaneously, the complexes bind to anti-rabbit-antibody coated to the microtiter plate. After washing, streptavidine conjugated to horseradish peroxidase (HRP) is added, which binds to the Biotin-Ab complexes. Free enzyme label is discarded and after a second washing step, Tetramethylbenzidine (TMB) Substrate is added to the wells. During the following incubation step, a colored product is formed in inverse proportion to the concentration of 1-MeTIQ present in the sample. Upon addition of Stop Solution the color changes from blue to yellow and can be read in a plate reader at 450 nm.

REAGENTS SUPPLIED AND PREPARATION

Reagents	Quantity	Code	Reconstitution
Microtiter Plate precoated with Goat–anti- rabbit polyclonal Ab	12 x 8 wells	B-MTQ-MP	Ready to use
Plate Sealer	3 pieces		
Wash Buffer Concentrate (10x) with preservatives	1 bottle 100 ml	B-MTQ-WB	Dilute with 900 ml of deionized water
Incubation Buffer with preservatives	1 bottle 100 ml	B-MTQ-IB	Ready to use
Calibrator 1-MeTIQ in a buffer with preservatives	1 vial	B-MTQ-CA	Ready to use
Control Low / High [1)] Lyophilized 1-MeTIQ within human serum	2 vials	B-MTQ-CONSET	Add 1 ml of Incubation Buffer
Antiserum Rabbit 1-MeTIQ Antibody in a buffer with preservatives	1 vial 5.5 ml	B-MTQ-AS	Ready to use
Biotin Conjugate 1-MeTIQ conjugated to biotin in a buffer with preservatives	1 vial 5.5 ml	B-MTQ-BC	Ready to use
Enzyme Label streptavidine conjugated HRP in a buffer with preservatives	1 vial 11 ml	B-MTQ-EL	Ready to use
TMB Substrate TMB in citrate buffer with H_2O_2	1 vial 11 ml	B-TMB	Ready to use
Stop Solution 0.25 M sulfuric acid	1 vial 11 ml	B-STS	Ready to use **Corrosive agent**

Table 1

1) The Controls contain lot-specific amounts of 1-MeTIQ. Refer to the QC Data Sheet for actual concentrations.

STORAGE AND SHELF LIFE OF REAGENTS

Unopened Reagents	
The unopened kit components are stable at 2-8 °C. Do not use the kit after the expiration date.	
Opened / Reconstituted Reagents	
Microtiter Plate	Return unused strips immediately to the aluminium pouch containing the desiccant packs and reseal along the entire edge of zip-seal. Stable at 2-8°C for 2 months
Controls	Stable at 2-8 °C for up to 4 months
Diluted Wash Buffer	Stable at 2-8°C until expiration date
Biotin Conjugate	
Antiserum	
Calibrator	
Incubation Buffer	
Enzyme Label	
Substrate Solution	
Stop Solution	Store at 18-28°C until expiration date

Table 2

WARNINGS AND PRECAUTIONS

The controls of this kit contain components of human origin. Each serum donor unit used in the preparation of the components was tested by an FDA approved method and found negative for HBV surface antigen, so as for HCV and HIV1/2 antibodies. Although these methods are highly accurate, there is no guarantee that this material cannot transmit Hepatitis or AIDS. *Therefore, patient samples and kit components should be handled as potentially infectious.* All products containing human source material should be handled in accordance with good laboratory practice and with appropriate precautions.

MATERIALS REQUIRED BUT NOT PROVIDED

Extraction Procedure:

- Extraction columns packed with Styroldivinylbenzol-polymer (Strata™, Phenomenex 8B-S100-UBJ, www.phenomenex.com)
- Extraction vacuum manifold
- Methanol p.a.
- 0.4 M perchloric acid
- Tissue homogenisator
- Disposable polysterene, polypropylene or glass tubes for the extraction

ELISA Procedure:

- Precision pipettes with disposable tips for 50 µl, 100 µl and 1000 µl
- Multishot pipettes with disposable tips for 50 µl and 100 µl.
- Disposable polystyrene or polypropylene tubes for preparation of calibrators and sample dilutions.
- 1000 ml cylinder for the dilution of the Wash Buffer concentrate
- Blotting Paper
- Microtiter Plate washer or squeeze bottle for the Wash Buffer
- Microtiter Plate shaker
- Microtiter Plate reader for measurement of absorbance at 450 nm

SPECIMEN COLLECTION AND STORAGE

1 ml serum, 1 g brain tissue or at least 50 µl CSF are needed to prepare the samples. **Serum and tissue samples have to be extracted according to the procedure described below. For CSF samples a simple dilution step is sufficient.**

Collect the specimen and store them at -20°C, if 1- MeTIQ will not be measured within a week.

SAMPLE PREPARATION

Cerebrospinal fluid (CSF)

Dilute sample 1:2 in Incubation Buffer (1 volume sample +1 volume Incubation Buffer).

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 ml 0.4 M perchloric acid.
4. Combine both supernatants and perform the solid phase extraction described below.

Extraction

Serum and brain tissue homogenates have to be extracted according to the following procedure. 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 H_2O
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 to collect the eluate.

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

7. Dilute 32% HCl 1:20 in methanol to obtain 0.5M HCl. Add 50 µl HCl 0.5 M in methanol to each eluate.
8. Evaporate samples under a constant nitrogen flow. Reconstitute samples by adding 1 ml incubation buffer. Let samples for 30 min at 37°C and treat them in an ultrasonic bath for 10min.

PROCEDURAL NOTES

The enzyme (HRP) is inactivated by oxygen and is highly sensitive to Sodium azide, Thimerosal, hypochlorous acid and aromatic chlorohydrocarbons often found in laboratory water supplies. Therefore, use only deionized or bistilled water.

If the concentration of an unknown sample reads an OD above the calibrator (10'000 pg/ml), the sample should be diluted with incubation buffer and re-assayed according to the assay procedure. The resulting dilution factor must be taken into account to calculate the final concentration.

ASSAY PROCEDURE

Important: Equilibrate the reagents to 18-28° C before performing the assay.

1. Calibrator dilution: Prepare a fresh standard curve for each assay being performed. The standard curve has to be prepared by serial dilutions of the Calibrator as follows:

   Label six tubes S1 to S6 and 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. Continue until the dilution series is completed. The corresponding concentrations of 1-MeTIQ are:

S1: 10'000 pg/ml	S4: 300 pg/ml
S2: 3'000 pg/ml	S5: 100 pg/ml
S3: 1'000 pg/ml	S6: 30 pg/ml

2. Prepare a frame with sufficient strips to test the desired number of samples. Remove excess strips from the frame and reseal them immediately in the foil pouch together with the desiccant packs. Store refrigerated.
3. Wash the coated wells twice with at least 300 µl Wash Buffer per well. Empty the wells and tap the plate firmly onto blotting paper.

4a. Pipet 50 µl of the Calibrator stock solution (1 µg/ml in duplicate into wells A1+A2 (Blank, NSB).

Pipet 50 µl of Incubation Buffer in duplicate into wells B1+B2 (Zero Calibrator).

Pipet 50 µl of diluted Calibrators S6 –S1 (30 pg/ml - 10'000 pg/ml) in duplicates into wells C1-H2.

4b. Pipet 50 µl of the Low Control into wells A3+A4.

Pipet 50 µl of the High Control into wells B3+B4.

4c. Pipet 50 µl of each processed sample in duplicates into the subsequent wells.

5. Pipet 50 µl Biotin Conjugate to all wells.
6. Pipet 50 µl Antiserum to all wells.
7. Cover the plate with a Plate Sealer and incubate at 18 - 28°C for 2 hours ± 5 min.
8. Remove and discard the Plate Sealer. Empty the wells and wash three times using at least 300 µl of Wash Buffer per well. Empty the wells and tab the plate firmly onto blotting paper.
9. Pipet 100 µl of Enzyme Label to all wells.
10. Cover the plate with a plate sealer and incubate for 1 hour ± 2 min. at 18-28 °C.
11. Remove and discard the plate sealer. Empty the wells and wash three times using at least 300 µl of Wash Buffer per well. Empty the wells and strike the plate firmly onto blotting paper.
12. Pipet 100 µl of the TMB Substrate to all wells.
13. Cover the plate with a Plate Sealer and incubate for 30 ± 1 min. at 18-28 °C in the dark.
14. Pipet 100 µl of Stop Solution to all wells. Remove air bubbles with a pipette tip. Proceed to step 15 within 30 minutes.
15. Read the absorbance at 450 nm in a microtiter plate reader.

RESULTS

Standard Curve

Record the absorbance at 450 nm for each calibrator and blank (NSB) well. Calculate the average of the duplicates, subtract the average of the blank and record averages (=corrected average absorbance). Calculate the binding (B) of each calibrator as percentage of the Zero Calibrator (B0), with the NSB-corrected absorbance of the Zero Calibrator taken as 100 %.

$$B/B_0\ (\%) = \text{percent bound} = \frac{\text{net absorbance}}{\text{net absorbance of Zero Calibrator}} \times 100$$

Plot the percent bound (vertical axis) versus the concentration of 1-MeTIQ in pg/ml (horizontal axis) using a lin/log graph. Draw the best fitting curve or calculate the standard curve using a four parameter logistic. Refer to Table 3 and Figure 1 for typical results. These are provided for demonstration purposes only. A standard curve must be generated along with each set of samples to be assayed.

Samples and Controls

Record the absorbance at 450 nm for each sample well. Average the duplicate values, subtract the average of the blank wells and record the averages (=corrected average absorbance). Calculate, as described above, the binding of each pair of sample wells as a percent of Zero Calibrator (B0), with the NSB-corrected absorbance of the Zero Calibrator taken as 100%. Locate the B/B0 value of the samples on the vertical axis, draw a horizontal line intersecting the standard curve and read the 1-MeTIQ concentration (pg/ml) from the horizontal axis.

STANDARDIZATION

The standard material consists of 1-Methyl-1,2,3,4-Tetrahydroisoquinoline calibrated with UV-Spectrometry.

QUALITY CONTROL

It is good laboratory practice to record the following data for each assay: kit lot number, reconstitution dates of kit components, results for Calibrators and Controls, and internal or controls.

The precision and expected values of standard curve and controls should be within established limits. The confidence limits for the controls are lot-specific and printed on the QC data sheet delivered with the kit.

Reliable results will only be obtained by using precise laboratory techniques (current GLP guidelines) and by accurate attention of this instruction for use.

If the precision of the assay is not within the established limits and repetition excludes errors in technique, check the following issues: i) pipetting, temperature controlling and timing devices ii) ELISA reader settings iii) expiration dates of reagents iv) storage and incubation conditions v) TMB Substrate Solution should be colorless vi) purity of water.

LIMITATIONS

The reagents supplied with this kit are optimized to measure 1-MeTIQ in serum samples, brain tissue and CSF.

The optimal incubation temperature is between 20 and 28 ℃ for Enzyme Label and TMB.

PERFORMANCE CHARACTERISTICS

Intra-Assay Precision: 8.7 %. The intra-assay precision was calculated from 20 pairs of values of 4 serum samples assayed in a single run according to the assay procedure. The results are presented in Table 4.

Inter-Assay Precision: 16.0 %. The inter-assay precision of the ELISA was calculated from 3 serum samples. The aliquots were assayed 18-20 times in 18-20 different runs according to the assay procedure. The values are presented in Table 5.

Dilution Linearity: Serum: 103 %; CSF: 98 %. Two spiked serum and CSF samples were serially diluted and assayed according to the assay procedure. For each serum, the expected value was calculated starting from the observed value nearest to the ED50. The results are presented in Table 6.

Spiking Recovery: Serum: 102 %; CSF: 102 %. Two serum and CSF samples were spiked with different amounts of 1-MeTIQ. The serum samples were extracted, CSF samples were diluted 1:2 in incubation buffer and measured before and after spiking according to the assay procedure. The results are presented in Table 7.

Analytical Sensitivity: ≤ 15.3 pg/ml. The minimal detectable dose of 1-MeTIQ was calculated to be 15.3 pg/ml by subtracting two standard deviations from the mean absorbance of the blank (Incubation Buffer) and intersecting this value with the standard curve obtained in the same run.

Functional Sensitivity: ≤ 100 pg/ml. Four spiked serum samples with values between 111 and 6.870 pg/ml 1-MeTIQ were assayed 20 times in duplicates as an intra-assay and 20 times in an inter-assay. The % CV and the mean values were calculated for each sample. The functional sensitivity at 15% CV for intra-assay and 20 % CV for inter assay was determined to be ≤ 100 pg/ml. According to the precision profile shown in Figure 2 the dynamic range of the assay is between 100 and 10'000 pg/ml.

Specificity: The substances listed in Table 8 have been measured to determine the cross reactivity. Cross-reaction of the anti-1-MeTIQ antibody has been determined at 50 % binding.

Table 3: Example of Results

	Absorb. (OD)	CV (%)	B/B0 (%)	Conc. (pg/ml)
Blank	0.069			
Cal 0	1.428		100.4	
Cal 0	1.417		99.6	
Cal 0 Avg.	**1.422**	**0.5**	**100.0**	0.0
Cal S6	1.393		97.9	
Cal S6	1.317		92.6	
Cal S6 Avg.	**1.355**	**4.0**	**95.3**	**30**
Cal S5	1.269		89.2	
Cal S5	1.143		80.2	
Cal S5 Avg.	**1.206**	**7.4**	**84.8**	**100**
Cal S4	0.942		66.2	
Cal S4	0.837		58.9	
Cal S4 Avg.	**0.889**	**8.3**	**62.5**	**300**
Cal S3	0.529		37.2	
Cal S3	0.446		31.4	
Cal S3 Avg.	**0.488**	**12.1**	**34.3**	**1'000**
Cal S2	0.247		17.4	
Cal S2	0.240		16.8	
Cal S2 Avg.	**0.243**	**2.3**	**17.1**	**3'000**
Cal S1	0.091		6.4	
Cal S1	0.098		6.9	
Cal S1 Avg.	**0.095**	**4.8**	**6.6**	**10'000**
Control low	0.888			
Control low	0.896			
Control low Avg.	**0.892**	**0.7**		**310**
Control high	0.290			
Control high	0.294			
Control high Avg.	**0.292**	**1.0**		**2'150**

ED-20 = 2.222pg/ml ED-50 = 516pg/ml ED-80 = 13 pg/ml

Figure 1: Example of Standard Curve

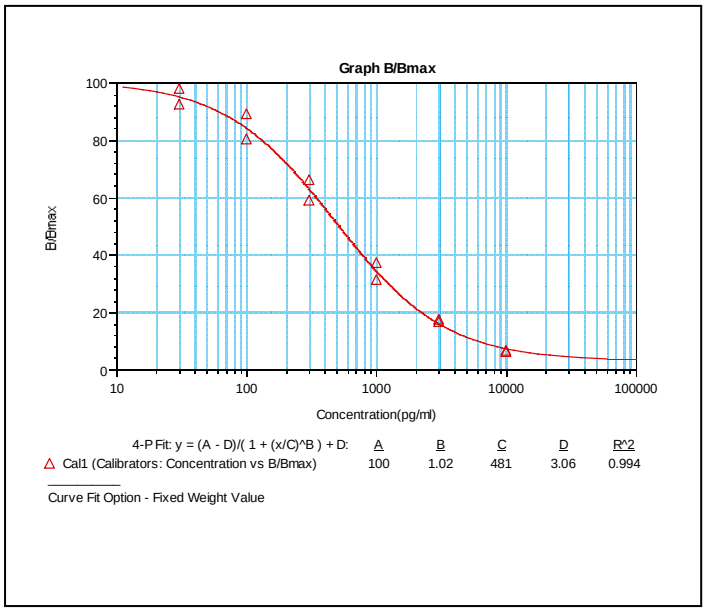

Table 4: Intra-Assay Precision

Sample spiked with 1-MeTIQ	Mean [pg/ml]	SD [pg/ml]	CV [%]
Serum1	132	18.1	13.7
Serum2	795	50.5	6.4
Serum3	3'720	288	7.7
Serum4	6'673	456	6.8
Mean			**8.7**

Table 5: Inter-Assay Precision

Sample spiked with 1-MeTIQ	Mean [pg/ml]	SD [pg/mL]	CV [%]
Serum1	702	86.8	12.4
Serum2	3'346	475	14.2
Serum3	6'873	1'462	21.3
Mean			**16.0**

Table 6: Dilution Linearity

Example 1 of 2	Dilution	Observed [pg/ml]	Expected [pg/ml]	O/E [%]
Serum1	-	8'322	7'187	116
	1:1.5	5'318	4'792	111
	1:2.3	3'047	3'194	95
	1:5	1'475	1'420	104
	1:7.6	952	947	101
	1:11	631	631	100
	1:17	387	421	92
	1:26	196	280	70
	1:38	131	187	70
Serum 1 mean				95
Serum 2 mean				110
Mean serum				**103**
CSF 1	1:2	3'844	3'736	103
	1:4	1'687	1'868	90
	1:8	929	934	99
	1:16	467	467	100
	1:32	270	234	116
	1:64	139	117	119
CSF1 mean				105
CSF2 mean				92
Mean CSF				**98**

Table 7: Spiking Recovery

Example 1 of 5	Native [pg/ml]	spiked with [pg/ml]	Observed [pg/ml]	Expected [pg/ml]	O/E [%]
Serum1	181	300	439	481	91.3
		900	831	1'081	76.9
		2'700	2'640	2'881	91.6
		8'100	9'800	8'281	118
Mean serum 1					95
Serum 2	191	300	531	491	108
		900	1'165	1'091	107
		2'700	3'090	2'891	107
		8'100	9'844	8'291	119
Mean serum 2					110
Mean serum					**102**
CSF1	52	300	448	352	127
		900	1'070	952	112
		2'700	2'794	2'752	102
		8'100	7'688	8'152	94
Mean CSF1					109
CSF2	38	300	368	319	115
		900	802	919	87
		2'700	2'494	2'719	92
		8'100	6'932	8'119	85
Mean CSF2					95
Mean CSF					**102**

Figure 2: **Functional Sensitivity**

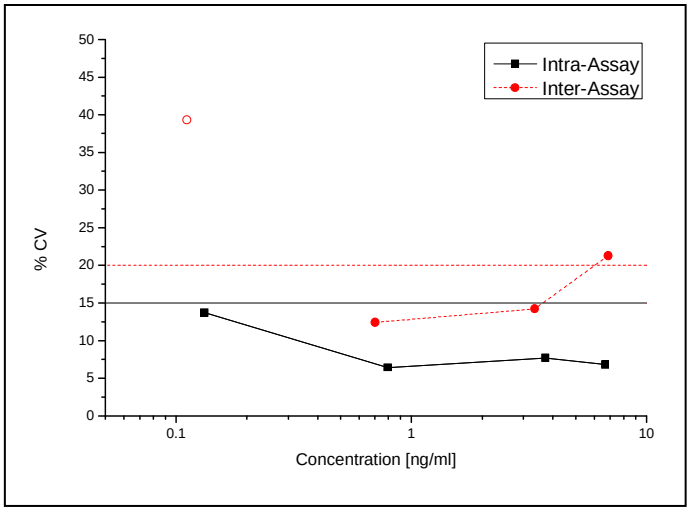

Table 8: **Cross Reactivity**

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 %
6,7-dimethoxy-3-methyl-1,2,3,4-tetrahydroisoquinoline HCl	< 1 %
1,2,3,4-tetrahydro-1-(phenylmethyl)-isoquinoline	< 1 %
1-methyl-4-phenylpyridinium iodide	< 1 %
1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine HCl	< 1 %
Haloperidol	< 1 %

APPENDIX II
REFERENCES/ LITERATURREFERENZEN/ REFERENCES/ RIFERIMENTI/ REFERENCIAS

1. Michell A.W. *et al.*: *Biomarkers and Parkinson's disease*. Brain **127,** 1693-1705 (2004)

2. Okuda K. *et al.*: *Determination method of 1-methyl-1,2,3,4-tetrahydroisoquinoline, an endogenous Parkinson-preventing substance, by radioimmuniassay.* Life Sci **70,** 2871-2873 (2002)

3. Ohta S. *et al.*: *Tetrahydroisoquinoline and 1-Methyl-Tetrahydroisoquinoline are Present in the Human Brain: Relation to Parkinson's Disease*. Biomedical Research **8,** 453-456 (1987)

PIPETTING PROTOCOL

1-MeTIQ ELISA

Precoated Microtiter Plate

wash 2 x

50 µl Calibrators, Controls
or Samples
+
50 µl 1-MeTIQ-Biotin-Conjugate
+
50 µl 1-MeTIQ -Antiserum

incubate 2 hours ± 5 min at 18-28°C
wash 3 x

add 100 µl Enzyme Label

incubate 1 hours ± 2 min at 18-28°C

wash 3 x

add 100 µl TMB Substrate

incubate 30±2 minutes at 18-28°C

add 100 µl Stop Solution

Read absorbance at 450 nm (within 30 minutes)

assay Time: 3 h 30 min

Symbol	Explanation	Symbol	Symbol
	Use By	[BC]	Biotin Conjugate
[REF]	Catalogue number	[AB]	Antiserum
[LOT]	Batch code	[EL]	Enzyme Label
	Sufficient for 96 tests	[SUBS|TMB]	TMB Substrate
	Consult Instructions for Use	[CAL]	Calibrator
	Temperature limitation	[CONTROL|L]	Control Low
[MP]	Microtiter plate	[CONTROL|H]	Control High
[BUF|WASH|10X]	Wash Buffer Concentrate (10x)	[SOLN|STOP]	Stop Solution
[BUF|INC]	Incubation Buffer		

Zertifiziertes Managementsystem SQS ISO 9001 / ISO 13485

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