Immusmol SAS
229 cours de l'argonne
33000 Bordeaux – France
+33 (0)5 6431 1170
contact@immusmol.com
www.immusmol.com

Instructions for use

L-Asparagine ELISA

REF **IS I-1600R**

+2 +8 °C

Σ 96

RUO

1. Intended use and principle of the test

Enzyme Immunoassay for the quantitative determination of L-Asparagine (ASN) in plasma samples.
After extraction and derivatization L-Asparagine is quantitatively determined by ELISA.
The competitive ELISA uses the microtiter plate format. The antigen is bound to the solid phase of the microtiter plate. The processed standards, controls and samples and the solid phase bound analyte compete for a fixed number of antiserum binding sites. When the system is in equilibrium, free antigen and free antigen-antiserum complexes are removed by washing. The antibody bound to the solid phase is detected by an anti-rabbit IgG-peroxidase conjugate using TMB as a substrate. The reaction is monitored at 450 nm.
Quantification of unknown samples is achieved by comparing their absorbance with a reference curve prepared with known standards.

2. Procedural cautions, guidelines, warnings and limitations

2.1 Procedural cautions, guidelines and warnings

(1) This kit is intended for professional use only. Users should have a thorough understanding of this protocol for the successful use of this kit. Only the test instruction provided with the kit is valid and has to be used to run the assay. Reliable performance will only be attained by strict and careful adherence to the instructions provided.
(2) This assay was validated for certain types of samples as indicated in *Intended Use* (please refer to Chapter 1). Any off-label use of this kit is in the responsibility of the user and the manufacturer cannot be held liable.
(3) The principles of Good Laboratory Practice (GLP) have to be followed.
(4) In order to reduce exposure to potentially harmful substances, wear lab coats, disposable protective gloves and protective glasses where necessary.
(5) All kit reagents and specimens should be brought to room temperature and mixed gently but thoroughly before use. Avoid repeated freezing and thawing of reagents and specimens.
(6) For dilution or reconstitution purposes, use deionized, distilled or ultra-pure water.
(7) The microplate contains snap-off strips. Unused wells must be stored at 2 °C to 8 °C in the sealed foil pouch with desiccant and used in the frame provided.
(8) Duplicate determination of sample is highly recommended to be able to identify potential pipetting errors.
(9) Once the test has been started, all steps should be completed without interruption. Make sure that the required reagents, materials and devices are prepared ready at the appropriate time.
(10) Incubation times do influence the results. All wells should be handled in the same order and time intervals.
(11) To avoid cross-contamination of reagents, use new disposable pipette tips for dispensing each reagent, sample, standard and control.
(12) A standard curve must be established for each run.
(13) The controls should be included in each run and fall within established confidence limits. The confidence limits are listed in the QC-Report.
(14) Do not mix kit components with different lot numbers within a test and do not use reagents beyond expiry date as shown on the kit labels.
(15) Avoid contact with Stop Solution containing 0.25 M H_2SO_4. It may cause skin irritation and burns. In case of contact with eyes or skin, rinse off immediately with water.
(16) TMB substrate has an irritant effect on skin and mucosa. In case of possible contact, wash eyes with an abundant volume of water and skin with soap and abundant water. Wash contaminated objects before reusing them.
(17) For information on hazardous substances included in the kit please refer to Safety Data Sheet (SDS). The Safety Data Sheet for this product is made available directly on the website of the manufacturer or upon request.
(18) The expected reference values reported in this test instruction are only indicative. It is recommended that each laboratory establishes its own reference intervals.
(19) The results obtained with this test kit should not be taken as the sole reason for any therapeutic consequence but have to be correlated to other diagnostic tests and clinical observations.
(20) Kit reagents must be regarded as hazardous waste and disposed of according to national regulations.

2.2 Limitations

Any inappropriate handling of samples or modification of this test might influence the results.
Samples containing precipitates or fibrin strands or which are hemolytic or lipemic might cause inaccurate results.

3. Storage and stability

Store the unopened reagents at 2 - 8 °C until expiration date. Do not use components beyond the expiry date indicated on the kit labels. Once opened the reagents are stable for 1 month when stored at 2 – 8 °C (except the BA E-2428 Equalizing Reagent, see 6.1). Once the resealable pouch has been opened, care should be taken to close it tightly with desiccant again.

4. Materials

4.1 Contents of the kit

BA D-0090 FOILS **Adhesive Foil** - Ready to use
Contents: Adhesive Foils in a resealable pouch
Volume: 1 x 4 foils

IS I-0324 REAC-PLATE **Reaction Plate** - Ready to use
Contents: 1 x 96 well plate, empty in a resealable pouch

BA E-0030 WASH-CONC 50x **Wash Buffer Concentrate** - Concentrated 50x
Contents: Buffer with a non-ionic detergent and physiological pH
Volume: 1 x 20 mL/vial, light purple cap

BA E-0040 CONJUGATE **Enzyme Conjugate** - Ready to use
Contents: Goat anti-rabbit immunoglobulins conjugated with peroxidase
Volume: 1 x 12 mL/vial, red cap

BA E-0055 SUBSTRATE **Substrate** - Ready to use
Contents: Chromogenic substrate containing tetramethylbenzidine, substrate buffer and hydrogen peroxide
Volume: 1 x 12 mL/black vial, black cap

BA E-0080 STOP-SOLN **Stop Solution** - Ready to use
Contents: 0.25 M sulfuric acid
Volume: 1 x 12 mL/vial, light grey cap
Hazards identification:

H290 May be corrosive to metals.

IS I-1631 L-PHE **Microtiter Strips** - Ready to use
Contents: 1 x 96 well (12x8) antigen precoated microwell plate in a resealable pouch with desiccant, silver bag

IS I-1610 AS L-PHE **L-Asparagine Antiserum** - Ready to use
Contents: Rabbit anti-L-Asparagine antibody, green coloured
Volume: 1 x 6 mL/vial, green cap

BA E-2413 ASSAY-BUFF **Assay Buffer** - Ready to use
Contents: Buffer with alkaline pH
Volume: 1 x 20 mL/vial, yellow cap

BA E-2428 EQUA-REAG **Equalizing Reagent** - Lyophilized
Contents: Lyophilized protein
Volume: 1 vial, brown cap

BA E-2446 D-REAGENT **D-Reagent** - Ready to use
Contents: Crosslinking agent in dimethylsulfoxide
Volume: 1 x 3 mL/vial, white cap

BA E-0429 RED-CONC 100x **Reducing Concentrate** – Concentrated 100X
Contents: Reducing agent in sodium hydroxide

Volume: 1 x 1 mL/vial, pink cap

Hazards identification :

H290 May be corrosive to metals.
H301 Toxic if swallowed.
H314 Causes severe skin burns and eye damage.
H360FD May damage fertility. May damage the unborn child

IS I-0488 PBS **PBS** - Ready to use
Contents: Phosphate Buffered Saline
Volume: 1 x 50 mL/vial, orange cap

BA E-2721 PREC-REAG **Precipitating Reagent** - Ready to use
Contents: Acidic reagent for precipitation of plasma/serum proteins, red coloured
Volume: 2 x 4 mL/vial, white cap

Standards and **Controls** - Ready to use

Cat. no.	Component	Colour/Cap	Concentration µg/mL	Concentration µmol/L	Volume/ Vial
IS I-1601	STANDARD A	white	0	0	4 mL
IS I-1602	STANDARD B	light yellow	1.3	9,6	4 mL
IS I-1603	STANDARD C	orange	3.2	24	4 mL
IS I-1604	STANDARD D	dark blue	7.9	60	4 mL
IS I-1605	STANDARD E	light grey	19,8	150	4 mL
IS I-1606	STANDARD F	black	49,5	375	4 mL
IS I-1651	CONTROL 1	light green	Refer to QC-Report for expected value and acceptable range!		4 mL
IS I-1652	CONTROL 2	dark red			4 mL

Conversion: L-Asparagine (µg/mL) x 7.57 = L-Asparagine (µmol/L)
Contents: Buffer with non-mercury stabilizer, spiked with defined quantity of L-Asparagine

4.2 Additional materials and equipment required but not provided in the kit

- Calibrated precision pipettes to dispense volumes between 10 – 300 µL; 12.5 mL
- Polystyrene or polypropylene tubes (0.5 mL) and suitable rack
- Microtiter plate washing device (manual, semi-automated or automated)
- ELISA reader capable of reading absorbance at 450 nm and if possible 620 - 650 nm
- Microtiter plate shaker (shaking amplitude 3 mm; approx. 600 rpm)
- Absorbent material (paper towel)
- Water (deionized, distilled or ultra-pure)
- Vortex mixer

5. Sample collection and storage

EDTA Plasma
Whole blood should be collected into centrifuge tubes containing EDTA as anti-coagulant (Monovette™ or Vacuette™ for plasma) and centrifuged according to manufacturer's instructions at room temperature immediately after collection.
Haemolytic and especially lipemic samples should not be used for the assay.
Storage: up to 48 hours at 2 - 8 °C, for longer period (up to 6 month) at -20 °C.
Repeated freezing and thawing should be avoided.

6. Test procedure

Allow all reagents and samples to reach room temperature and mix thoroughly by gentle inversion before use. Duplicate determinations are recommended. To do that, perform duplicates after derivatization (1 derivatization well = 2 competition wells).
It is recommended to number the strips of the microwell plate before usage to avoid any mix-up.

The binding of the antisera and of the enzyme conjugate and the activity of the enzyme are temperature dependent, and the absorbance values may vary if a thermostat is not used. The higher the temperature, the higher the extinction values will be. Corresponding variations also apply to the incubation times. The optimal temperature during the Enzyme Immunoassay is between 20 – 25 °C.

6.1 Preparation of reagents

Wash Buffer
Dilute the 20 mL Wash Buffer Concentrate with water (deionized, distilled or ultra-pure) to a final volume of 1000 mL.
Storage: 1 month at 2 – 8 °C

Equalizing Reagent
Reconstitute the Equalizing Reagent with **12.5 mL** of **Assay Buffer**.
Reconstituted Equalizing Reagent which is not used immediately has to be stored in aliquots for max 1 month at -20 °C and may be thawed only once.

D-Reagent
The D-Reagent has a freezing point of 18.5 °C. It must be ensured that the D-Reagent has reached room temperature and forms a homogeneous, crystal-free solution.

Reducing Solution
Dilute Reducing Concentrate 1:100 with water (deionized, distilled, or ultra-pure) and mix thoroughly. Use immediately!

Examples for the preparation of Reducing Solution:

Reducing Concentrate	40 µL	50 µL	80 µL	160 µL
Water	3.96 mL	4.95 mL	7.92 mL	15.84 mL

L-Asparagine Microtiter Strips
In rare cases residues of the blocking and stabilizing reagent can be seen in the wells as small, white dots or lines. These residues do not influence the quality of the product.

6.2 Precipitation

1.	Pipette **20 µL** of **standards**, **controls** and **samples** into the respective tubes.
2.	Add **50 µL of PBS** to all tubes.
3.	Add **10 µL Precipitating Reagent** to all tubes.
4.	Mix the **tubes** thoroughly (vortex) and centrifuge for **15 minutes** at **3000 x g**.
⚠	Take **25 µL** of the clear supernatant for the **derivatization**.

6.3 Derivatization

1.	Pipette **25 µL** of the **precipitated standards, controls** and **samples** into the appropriate wells of the **Reaction Plate.**
2.	Add **50 µL** of the **Equalizing Reagent** into all wells.
3.	Mix 2 min at RT on a plate shaker at 700 rpm to homogenize the medium.
4.	Add **10 µL** of the **D-Reagent** into all wells (immerse the tips into the reaction medium).
5.	Cover plate with **Adhesive Foil** and incubate for **2 h** at **RT** (20 – 25 °C) on a **shaker** (approx. 500 rpm).
6.	Prepare **Reducing Solution 1X** from Reducing Concentrate 100X **in sterile water** (see 6.1) ***The Reducing Solution should be prepared directly prior to use!***
7.	Add **100 µL** of the **Reducing Solution 1X** into all wells.
8.	Incubate for **10 min** at **RT** (20 – 25 °C) on a **shaker** (approx. 500 rpm).
⚠	**Use 25 µL for the ELISA!**

6.4 L-Asparagine ELISA

1.	Pipette **25 µL** of the **prepared standards, controls and samples** into the appropriate wells of the **L-Asparagine Microtiter Strips.** ***Be careful not to pipette bubbles!***
2.	Add **50 µL** of the **L-Asparagine Antiserum** into all wells and mix shortly.
3.	Cover plate with **Adhesive Foil** and incubate for **15 - 20 h** (overnight) at **2 – 8 °C**.
4.	Remove the foil. Discard or aspirate the content of the wells. Wash the plate **4 x** by adding **300 µL** of **Wash Buffer, discarding** the content and **blotting dry each time** by tapping the inverted plate on absorbent material.
5.	Pipette **100 µL** of the **Enzyme Conjugate** into all wells.
6.	Incubate for **30 min** at **RT** (20 – 25 °C) on a **shaker** (approx. 500 rpm).
7.	Discard or aspirate the content of the wells. Wash the plate **4 x** by adding **300 µL** of **Wash Buffer, discarding** the content and **blotting dry each time** by tapping the inverted plate on absorbent material.
8. ⚠	Pipette **100 µL** of the **Substrate** into all wells and incubate for **15 - 25 min** at **RT** (20 – 25 °C) on a **shaker** (approx. 500 rpm). ***Avoid exposure to direct sunlight!***
9.	Add **100 µL** of the **Stop Solution** to each well and shake the microtiter plate to ensure a homogeneous distribution of the solution.
10.	**Read** the **absorbance** of the solution in the wells within 10 minutes, using a microplate reader set to **450 nm** (if available a reference wavelength between 620 nm and 650 nm is recommended).

7. Calculation of results

Measuring range	**L-Asparagine**
	9,6 – 375 µM

The calibration curve is obtained by plotting the absorbance readings (calculate the mean absorbance) of the standards (linear, y-axis) against the corresponding standard concentrations (logarithmic, x-axis).
Use non-linear regression for curve fitting (e.g. spline, 4- parameter, akima).

⚠ *This assay is a competitive assay. This means: the OD-values are decreasing with increasing concentrations of the analyte. OD-values found below the standard curve correspond to high concentrations of the analyte in the sample.*

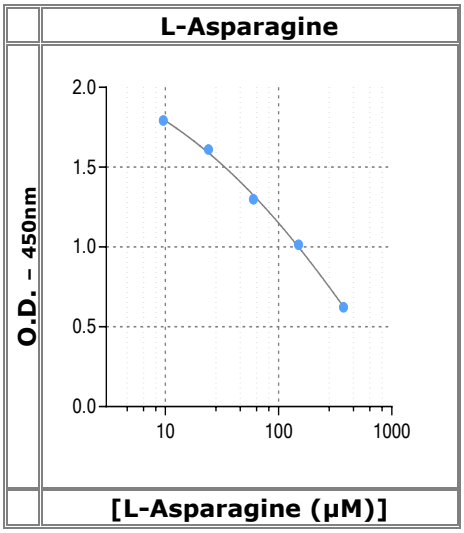
The concentrations of the samples and controls can be read directly from the standard curve.

7.1 Quality control

The confidence limits of the kit controls are indicated on the QC-Report.

7.2 Typical standard curve

⚠ *Example, do not use for calculation!*



8. Assay characteristics

Analytical Sensitivity (Limit of Detection)	**L-Asparagine**
	2,3 µM

Analytical Specificity (Cross Reactivity)	**Substance**	**Cross Reactivity (%)**
	L-Asparagine	100%
	D-Asparagine	0,1%
	L-Aspartate	<0,2%
	L-Glutamate	<0,1%
	L-Glutamine	0,1%

Intra-Assay			
Plasma sample	Mean (µM)	SD (µM)	CV (%)
1 (n = 8)	61	5	7
2 (n = 8)	113	4	4
3 (n = 8)	171	17	10

Linearity	Plasma sample (Serial dilution up to 1:64)	Range Linearity (%)	Mean Linearity (%)
	1	107 - 124	112
	2	105 - 116	109
	3	91 - 111	100

Recovery	Plasma sample	Range Recovery (%)	Mean Recovery (%)
	1	93 - 101	98
	2	93 - 104	99
	3	92 - 108	100

Method Comparison: ELISA vs LC-MS	Plasma Sample	$[\text{L-Asparagine}]_{ELISA} = 1{,}034 * [\text{L-Asparagine}]_{LC\text{-}MS} + 2{,}919$	$R^2 = 0{,}9774$ N = 40

⚠ *For updated literature or any other information please contact your local supplier.*

Symbols:

	Storage temperature		Manufacturer		Contains sufficient for <n> tests
	Expiry date	LOT	Batch code	RUO	For research use only!
	Consult instructions for use	CONT	Content		
	Caution	REF	Catalogue number		