Product Description

Anti-Phenanthrene IgG (Mouse) ELISA Kit

Intended Use

Anti-Phenanthrene IgG (Mouse) ELISA Kit is an ELISA kit intended for a qualitative detection of anti-Phenanthrene IgG antibodies in mouse serum.

Test Principle

Anti-Phenanthrene IgG (Mouse) ELISA Kit is a solid-phase immunoanalytical test. Microtiter wells are coated with Phenanthrene protein conjugate. If there are antibodies to Phenanthrene present in a serum sample, they will bind to the immobilised Phenanthrene protein conjugate. The bound antibodies react in the next step with animal anti- mouse IgG antibody labelled with horseradish peroxidase. The amount of the bound labelled antibodies is determined by enzymatic reaction with a chromogenic substrate. Negative sera do not react, a mild change in colour, if present, is attributed to the reaction background.

Kit Components

8-well break-away strips coated with Phenanthrene protein conjugate (colourless) within a plastic frame		96 wells
1.3 mL	High positive control serum (HPCS), r.t.u.*	1 vial
1.3 mL	Middle positive control serum (MPCS), r.t.u. *	1 vial
1.3 mL	Low positive control serum (LPCS), r.t.u.*	1 vial
0.2 mL	Px-conjugate concentrate (animal anti- mouse IgG antibodies labelled with horseraddish peroxidase), 301x concentrated	1 vial
125 mL	Wash buffer concentrate, 10x concentrated	1 vial
125 mL	Dilution buffer (DB), r.t.u.*	1 vial
15 mL	Chromogenic substrate (TMB substrate), r.t.u.*	1 vial
30 mL	Stop solution, r.t.u.*	1 vial
Sealable pouch for unused strips		1 piece
Instruction manual		
Certificate of quality		

*(ready to use)

Material Required but Not Provide With the Kit

a. Distilled or deionised water for dilution of the Wash buffer concentrate.
b. Appropriate equipment for pipetting, liquid dispensing and washing.
c. Spectrophotometer/colorimeter (microplate reader – wavelength 450 nm).

Preparation of Reagents and Samples

a. **Allow all kit components to reach room temperature.**

b. **Vortex serum samples and the controls in order to ensure homogeneity** and mix all solution well prior use. **Dilute serum samples 1:100 in dilution buffer (DB)** – e.g. 5 µL of serum sample + 500 µL of dilution buffer. Do not dilute the controls – they are ready to use (r.t.u.).

c. Prepare Wash buffer by diluting the concentrate 10 times with an appropriate volume of distilled or deionised water (100 mL of the concentrated Wash buffer + 900 mL of distilled water). If there are crystals of salt present in the concentrated Wash buffer, warm up the vial to 32 to 37°C in a water bath. Diluted Wash buffer is stable for one week if stored at 2 to 10°C.

d. **Dilute concentrated Px-conjugate 1:300 with Dilution buffer (DB)** – e.g. 0.04 mL of Px-conjugate + 12 mL of Dilution buffer (DB). (Note: For one microtitre plate you will need approx. 12 mL of diluted Px-conjugate.)

e. Do not dilute TMB substrate and Stop solution, they are ready to use.

Assay Procedure

a. Allow the coated strips to reach room temperature before opening to prevent water condensation within the wells. Withdraw an adequate number of antigen coated strips. Put the remaining strips back in the provided pouche and seal it, keep the desiccant inside.

b. Pipette 100 µL of Dilution buffer, the controls and diluted serum samples to the wells according to the pipetting scheme in Figure 1: Start with filling the first well with Dilution buffer to estimate the reaction background, fill the next three wells with control serums – one well with the High positive control serum (HPCS), one with the Middle positive control serum (MPCS) and one with the Low positive control serum (LPCS). Fill the remaining wells with the diluted serum samples (S1, S2, S3,...). It is satisfactory to apply samples as singlets, however, if you want to minimise the laboratory error then apply the Controls and samples in doublets.

c. Incubate for **60 (+/- 5) minutes** at room temperature.

d. Aspirate the liquid from the wells into a collecting bottle containing appropriate disinfectant (*see Safety Precautions*). Wash and aspirate the wells five times with 250 µl/well of Wash buffer. Avoid cross-contamination between wells! If some liquid remains in the wells, invert the plate and tap it on an adsorbent paper to remove any remaining drops.

e. Add 100 µL of the diluted Px-conjugate into each well.

f. Incubate for **60 (+/- 5) minutes** at room temperature.

g. Aspirate and wash the wells five times with 250 µl/well of Wash buffer as in step “d”.

h. Dispense 100 µL of the TMB substrate into each well. *Pipette in a regular rhythm or use an appropriate dispensing instrument.*

i. Incubate for **10 minutes (+/-5 seconds)** at room temperature. **The time measurement must be started at the beginning of TMB dispensing.** Cover the strips with an aluminium foil or keep them in the dark during the incubation with TMB substrate.

j. Stop the reaction by adding 100 µL of Stop solution. Use the same pipetting rhythm as with the TMB substrate to ensure the same reaction time in all wells. Tap gently the microplate for a few times to ensure complete mixing of the reagents.

k. Read the absorbance at 450 nm with a microplate reader **within 10 minutes**. It is recommended to use a reference reading at 620-690 nm.

Figure.1.: Pippetting scheme

	1	2	3	4	5	6	7	8	9	10	11	12
a	DB											
b	HPCS											
c	MPCS											
d	LPCS											
e	S1											
f	S2											
g	S3											
h	S…											

Processing of Results

a. First subtract the absorbance of the background (absorbance of the DB well) from the absorbances of all other wells.

b. Calculate the ratio of the observed sample absorbance to the absorbance of the high positive control serum, **the Classification Index (CI).** Calculate the CI for the middle positive control serum and for the low positive control serum and for each serum sample by dividing the absorbances with high positive control serum (HPCS) absorbance:

$$\text{Classification Index} = \frac{\text{MPCS or LPCS or an experimental serum absorbance}}{\text{High positive control serum (HPCS) absorbance}}$$

Example:

Absorbances of High positive control serum (HPCS): = 1.115; 1.114

HPCS mean: = 1.115

Absorbances of the two parallels of a serum sample: = 0.814; 0.870

Sample mean: = 0.842

Classification Index: = 0.842/1.115 = 0.76

Value of the Classification Index	Interpretation
< 0.5	serum with low level of anti-Phenanthrene antibodies
> 0.5	serum with high level of anti-Phenanthrene antibodies

Validity, Specificity and Sensitivity of the Test

A. Validity of the test

The test is valid if:

a. The background of the reaction (the absorbance of the Dilution buffer) is less than 0.050.

b. The Classification Index of the Hight positive control serum is within the range 0.8-1.0.

c. The Classification Index of the Middle positive control serum is within the range 0.4-0.5.

d. The Classification Index of the Low positive control serum is within the range 0.1-0.2.

e. The absorbances of the Controls can be lined up as follows: LPCS < MPCS < HPCS.

B. Precision of the test

The intraassay variability (within the test) and the interassay variability (between tests) evaluation was performed with samples of variable absorbance values.

1. Intraassay variability

(N = number of parallels, SD = standard deviation):

N	Absorbance	SD	CV%
16	0.324	0.009	2.8 %
16	0.162	0.004	2.2 %

2. Interassay variability

(N = number of paralells, SD = standard deviation):

N	Absorbance	SD	Range (min-max)	CV%
14	0.284	0.030	0.235 – 0.346	10.6%
16	0.515	0.056	0.431 – 0.613	10.8%
16	0.786	0.089	0.674 – 0.971	11.3%
20	1.085	0.101	0.913 – 1.260	9.3%

Safety Precautions

All ingredients of the kit are intended for laboratory use only.

The Controls contain human sera that has been tested negative for HBsAg, anti-HIV-1,2 and anti-HCV. However, they should be regarded as contagious and handled and disposed of according to the appropriate regulations. Autoclave all reusable materials that were in contact with mouse samples for 1 hour at 121℃, burn disposable ignitable materials, decontaminate liquid wastes and nonignitable materials with 3% chloramine.

Liquid wastes containing acid (Stop solution) should be neutralized in 4% sodium bicarbonate solution. Handle Stop solution with care. Avoid contact with skin or mucous membranes. In case of contact with skin, rinse immediately with plenty of water and seek medical advice.

Do not smoke, eat or drink during work. Do not pipette by mouth. Wear disposable gloves while handling reagents or samples and wash your hands thoroughly afterwards. Avoid spilling or producing aerosol.

Handling Precautions

Avoid contamination of samples and kit reagents.

Avoid cross-contamination of reagents.

Control serums, dilution buffer (DB) and chromogenic substrate (TMB substrate) contain preservative ProClin 300®.

Avoid contact of the TMB substrate with oxidizing agents or metal surfaces.

Follow the assay procedure indicated in the Instruction manual.

Variations in test results are usually due to:

* Insufficient mixing of reagents and samples
* Inaccurate pipetting and inadequate incubation times
* Poor washing technique or spilling the rim of well with sample or Anti- mouse IgG Px-conjugate
* Use of identical pipette tip for different solutions

Storage and Expiration

Store the kit reagents at 2 to 10℃, in a dry place and protected from the light. Avoid freezing.

Expiration date is indicated at the ELISA kit label and at all reagent labels.

Store unused strips in the sealable pouch and keep the desiccant inside. The kit is transported in thermo bags and the transport time up to 72 hours have no influence on the kit performance. If you find any damages to the kit components please advise the producer.

Do not store diluted serum samples or diluted Px conjugate. Always prepare fresh.

Flow Chart

Step 1.	**Prepare reagents and samples**
	↓
Step 2.	**Add 100 µL/ well of the Controls and serum samples**
	↓
	Incubate 60 minutes at room temperature
	↓
	Wash 5 times (250 µL/well), aspirate
	↓
Step 3.	**Add 100 µL/ well of Px-conjugate**
	↓
	Incubate 60 minutes at room temperature
	↓
	Wash 5 times (250 µL/well), aspirate
	↓
Step 4.	**Add 100 µL/well TMB substrate**
	↓
	Incubate 10 minutes at room temperature
	↓
Step 5.	**Add 100 µL/well of Stop solution**
	↓
Step 6.	**Read optical density for 450 nm (ref. 620-690nm) till 10 min**