Diaproph Med

GENERAL RECOMMENDATIONS FOR ACCURATE PERFORMANCE OF ENZYME – LINKED IMMUNOSORBENT ASSAY

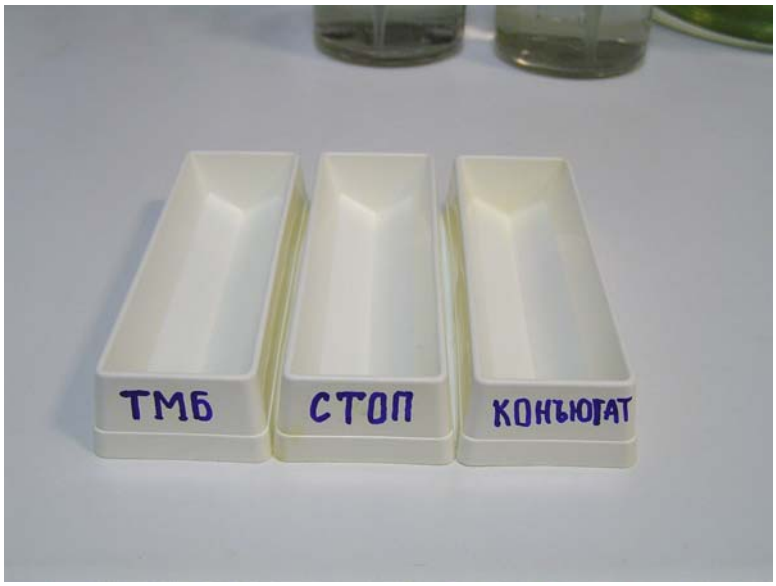
An accurate assay performance can be reached by obligatory observance the following requirements

- Quality treatment/preparation of laboratory glassware and distilled water
- Proper treatment of investigation material
- Correct handling of automatic pipette
- Following procedures according to the instruction for use and conditions of ELISA performance:
    1. Following temperature conditions and incubation time
    2. Quality washing of microplates at all phases of ELISA performance
    3. Accurate functioning of equipments – pipettes, washers, thermostats/incubators and ELISA readers

Recommendations for quality treatment of laboratory glassware for ELISA performance

- Glassware is to be washed using liquid stuff that does not contain biocomponents
- After washing the glassware should be thoroughly rinsed with running water then with distilled water
- It is important to use different containers for working with a conjugate solution and a developer solution
- For handling with conjugate and developer solutions, disposal tips are to be used

Different containers for working with conjugate and developer solution should be used during ELISA performance



Recommendations for treatment of investigated samples

- Prior to investigation in ELISA, a serum is allowed to keep for 72 hours in a fridge at 2-8°C
- If there is no possibility to investigate a serum or plasma during above mentioned time, sample should be frozen at -20°C or below.

- Serum samples are to be transparent, without hematolysis, hyperlipemia and traces bacteriemia,
- For obtaining of correct results at investigation of sera in dynamics of disease it is necessary to create bank for sera of initial sampling that can be stored in frozen conditions at -20°C and below

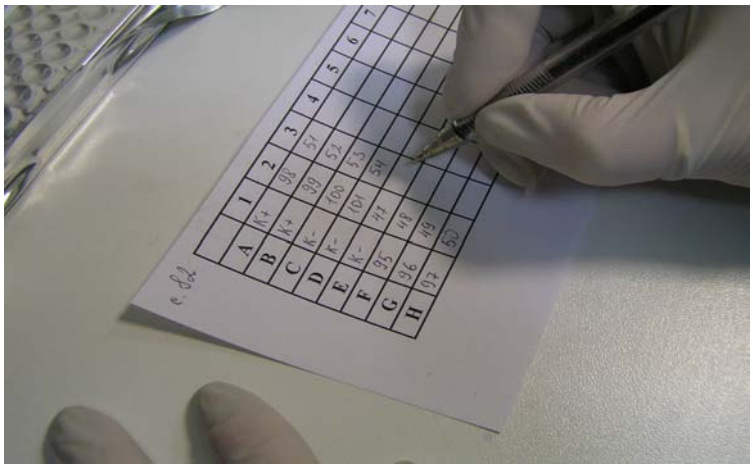
Conditions of ELISA performance

- During ELISA performance the temperature in laboratory room should be supported within 18-25°C.
- It is prohibited a presence in the laboratory of any vapour of oxidizing agents (open containers with solutions of hydrogen peroxide, hypoclorides etc.) and chlorinated solutions.
- During ELISA performance, the quality distilled water is to be used that should be kept no longer than two days.
- It is prohibited to use microplates of the kit for any other work than ELISA performance.
- The quality of diagnostics kits is guaranteed only within declared shelf life and accurately following conditions of storage for test kits.

Conditions of ELISA performance

- It is necessary to limit hitting of direct sun lights/rays on wells.
- When investigated samples are distributed and there is occurred error, e.g. two sera were put into the same well, such well is to be rejected. And in the distribution chart of samples there is marked the well, which is wasted.
- The time of filling of all required wells on the plate is not to be exceeded 15-20 minutes.

Prior to ELISA performance, the protocol of investigation should be filled in.



When you work with automatic pipette the following rules should be considered:

- Fix the required volume as indicated in the instruction for use for the pipette.
- To be sure that tips are clean and there is no strange plastic particles inside. The tip should be put closely together on the cone of pipette.
- During work handle the pipette upright (the maximum deviation from vertical line is 10°)
- The thumb should put on the operational button of the pipette.
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The sequence of performance when investigated samples distributing into solution for specimen preparation:

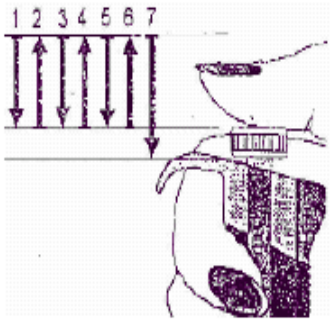
- Place the tip in the biological liquid down 2-3 mm and put the operational button up to the first stop
- Do not pull out the tip from liquid and carefully release the button in the initial position
- Place the tip in the liquid down 2-3 mm where is necessary to carry filled liquid, then put on the operational button up to the first stop

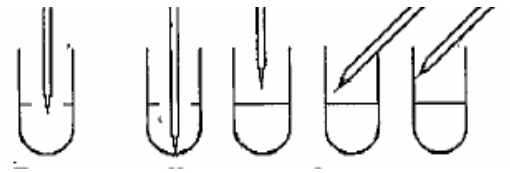
- In the position when the tip a little immersed in the solution then smoothly release the button (until its initial position). In such way the tip is filled for fluid mixture. Repeat this procedure 2-3 times.
- Then put on the operational button up to the second stop for complete releasing the tip from the liquid. Pull out the tip from the solution and release the operational button in the initial position.
- Remove the tip from the pipette with the mechanism for removing of tips.
- Change the tip and continue your work with the next sample of biological liquid

Initial position

First stop

Second stop





Correct position of the pipette

Wrong position of the pipette

For better observance for component distribution it is desirable to place the immunosorbent on a white background.

When you work with 8-chanel pipette it is should be paid attention to a uniformity of filling with liquid of all pipette tips.

Following temperature conditions and incubation time

- Filling time of all required wells is not to be longer than 15-20 minutes
- Incubation of plate at all ELISA phases is conducted at the temperature indicated in the kit insert and the room temperature is considered 18-25°C
- It is not allowed to incubate plates in the incubator putting them in a pile, since inhomogeneous warming of the plate in that case may cause to "edge"-effect – increased background around the periphery of the plate.

Washing of the plate at each phase of ELISA performance:

- **<u>The quality of plate washing – the one of determinative factor during ELISA performance</u>**
- The regime of washing is to be strictly followed according to requirements in the kit insert
- It is necessary to check an uniformity of filling of all wells and check there is no spilling washing solution over wells (from the one to the another)
- Incubation time with washing solution at each phase of washing should be not less than 30 seconds if there is not other requirements in the kit insert.
- For prevention of washer spoiling it is to be daily rinsed with distilled water and once a week with 30 % ethyl alcohol.
- The most frequent source of errors it is spoiling of channels in the washer or spaces between filling needles with salt crystals or else.

The developer solution directly before use should be transparent and colourless. Remains of solution that rested in the container it is recommended not to discarded the one until incubation termination of the plate with the developer. The developer solution that rests in the container does not change a colour during 30 minutes. And an appearance of coloration in the container is an evidence of solution contamination.

Periodical control of equipment functioning

It is important factor affecting to ELISA performance and allowing to have correct results is the **accuracy of equipment functioning:**

- **Automatic pipettes,**
- **Incubators,**
- **ELISA readers,**
- **Washers**

Troubleshooting can occur during ELISA performance

Problems that can occur	***Probable causes***	***Methods of elimination***
High background in wells all over the microplate	*Contaminated washer*	1. Clean a manifold / needle of the washer and wash with 30 % solution of ethyl alcohol then distilled water
	Poor quality water or contaminated water	1. Wash a distiller with 10-% solution of hydrochloric acid then 5 times with distilled water 2. Use bi-distilled water 3. Store distilled water in closed vessels not longer than 2 days
	Using of poor cleaning laboratory ware	1. Use for reagents separate laboratory ware, daily rinse with distilled water and weekly with a chromic mixture or detergents
High background in all wells of the microplate	*Presence and application at work areas of disinfectant solutions containing chlorine*	1. Do not use and do not store such disinfectant solutions
	Using contaminated tips	1. Use clean tips (for conjugates and substrates – disposal)
	Extended time of incubation or changed temperature conditions	1. Follow incubation regime according to the kit insert
High background in separate rows	*Repeated dispensing of a developer solution*	1. The developer solution is to be put once
	Contamination of tips of automatic pipette with a conjugate solution	1. Clean a pipette and carefully collect liquid, avoid “inleakage” of a solution 2. Use separate pipettes for conjugate
	Contaminated one of washer channels	1. Clean a channel of the washer and rinse the washer
Frequent «outliers» (irreproducible results) on the microplate	*Contaminated washer*	1. Clean a manifold / needle of the washer and wash with 30 % solution of ethyl alcohol then rinse with distilled water
No colouring in wells of the whole microplate (failure of kit controls)	*One of reagents was not correctly prepared or not put: a conjugate or chromogen*	1. Repeat ELISA performance, pay attention to reagent preparation

Problems that can occur	*Probable causes*	*Methods of elimination*
Weak colouring of the microplate (positive controls are lower then validation criteria in the kit insert)	*One of reagents was not correctly prepared or not put: a conjugate or chromogen*	1. Repeat ELISA performance and pay attention to reagent preparation
	Incubation time with chromogen was reduced	1. Incubation is to be in accordance with the kit insert