ELISA

Tips

Washing
Pipetting
Microplate
Temperature
Incubation

ELISA Tips

- Enzyme-linked immunosorbent assays (ELISAs) are plate-based assays designed for detecting and quantifying substances such as peptides, proteins, antibodies, and hormones.
- The reagents needed to construct and run a typical sandwich ELISA or other plate-based immunoassay include specific antibodies (e.g., matched antibody pairs), enzyme conjugates, blocking buffers, sample and wash buffers, and enzyme substrates.

Washing

- The purpose of washing is to separate bound and unbound (free / unwanted) reagents/serum components. This involves the emptying of microwells of reagents followed by the addition of liquid into the wells. Such a process is performed at least 3-6 times for every well. The liquid used to wash wells is usually buffered (PBS) in order to maintain isotonicity, since most Ag-Ab reactions are optimal under such conditions. Tap water is not recommended, since tap water varies greatly in composition (pH, molarity, and so on). Generally, the mechanical action of flooding wells with a solution is enough to wash wells of unbound reagents. Some workers leave washing solution for a short time (soak time) after each addition (1-5 minutes).

Washing

Sometimes detergents, notably Tween-20 (0.05%) are added to washing buffers. This can cause problems where excessive frothing takes place producing poor washing conditions, since air is trapped and prevents the washing solution from contacting the well surface. For most cases, this addition does not contribute significantly to the washing procedure. When using detergents, care has to be taken that they do not affect reagents adversely (denature Ag), and greater care is needed to prevent frothing in the wells.

Washing

NORMAL WASHING

- In washing plate manually, the most important factor is that each well receives the washing solution so that, no air bubbles are trapped in the well or a thumb is not placed over corner wells.

STRIP / PLATE WASHERS

- Various washing cycles can be programmed. Careful maintenance is essential, since they are prone to machine errors, such as having a particular nozzle being blocked. Further ELISA tips in detail will be recommended in the following aspects.

ELISA Tips on Washing

Calibrate pipettes regularly according to manufacturer's instructions.
2. Avoid touching side wall of well with tips.
3. Avoid splashing of sample and reagents.
4. Avoid blowing out tip contents.
5. Use a new tip for each sample/control/reagent addition.
6. New tips should be used on the multichannel pipettes for each reagent to be added.
7. Reverse pipette when using the multichannel pipettes to add conjugate and substrate solution.
8. Forward pipette when using the multichannel pipettes to add stop solution.
9. Check pipette tips are long enough to provide air space between top of tip and pipette barrel.
10. Check pipette barrel for residual fluid of dried material, remove if present.
11. Ensure pipettes tips are fitted tightly.

ELISA Tips on Pipetting

1. Calibrate pipettes regularly according to manufacturer's instructions.
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ELISA Tips on Microplate

1. Bring microplate pouches to room temperature before opening.
2. Level microwells evenly in microplate frame as the individual breakaway wells have very flexible plate frames leading to bowing off wells and yield poor washes.
3. Place plates in dark immediately after addition of substrate solution, provided the substrate is sensitive to light.
4. Grasp holder on grip marks when tapping to avoid strips slipping from holder.
5. Rotate strips 180 and re-insert or use correct holder if strips do not fit in holder.
6. Seal unused wells in purchase along with the desiccant.
7. Date the pouches when first opened.
8. Clean bottom surface of plates with wash buffer to remove fingerprints.
9. Make sure microwells are at level during washing, reagent addition and plate/strip reading.
10. Wipe the bottom the plate with a lint-free cloth/ towel before reading.
11. Do not allow microwells to become dry once the assay has begun

ELISA Tips on Temperature

1. Bring test reagents to room temperature (22-28 C) approximately 30 minutes prior to use.
2. Maintain proper incubation temperature:
   Lower temperature can decrease OD values.
   Higher temperatures can increase OD values.
   Evaporation in wells can cause edging effect.
3. The optimal temperature for incubation is 22-28 C.
4. Check temperature against calibrated thermometer.
5. Strict adherence to time must be maintained.
6. Check calibration of timers.
7. Record time of incubation.
8. Read plate with specified time limits of adding stop solution.

ELISA Tips on Incubation

1. Rotation of plates while incubating reagents.
2. In certain ELISA systems, the plates are rotated during incubation for better antigenantibody reaction.
3. The effect of rotating plates is to mix the reactants completely during the incubation step.
4. Since the solid-phase limits the surface area of the absorbed reactant, the mixing ensures that, potentially reactive molecules are continuously coming into contact with the solid-phase.
5. During stationary incubation, mixing only takes place because of diffusion of reagents.
6. Thus, to allow maximum reaction from reagents in stationary conditions, greater times of incubation may be required, than if they are rotated.
7. Rotation also allow ELISA to be performed independent of temperature conditions.
8. The interaction of antigen & antibodies relies on their closeness, and the kinetic energy provided to the system, which is encouraged with the mixing during rotation.
9. Stationary incubation relies on the diffusion of molecules & thus is dependent on temperature.

ELISA Tips on Others

- **Substrate Preparation**

  Use freshly prepared substrate A and substrate B (in 2-reagent substrate systems)
  Do not hold substrate solution longer than 1 hour.
  Follow procedure of working substrate solution.
  The temperature of solution is important because it effect s rate of colour reaction.
  Do not add fresh substrate to reagent bottle containing old substrate.
  Clean old substrate solution bottle with H SO and thoroughly rinse with distilled water.

- **Conjugates**

  Store at recommended temperature.
  Never store excessively diluted conjugate for use at some later time.
  Always make up the working dilution of conjugate just before you need it.
  Never leave conjugate on the bench for excessive time.

ELISA Tips on Others

- **Addition of Samples**

  Problems are usually caused by failure to put sample into buffer in well, leaving it on the side of the plate. Pay attention to the proper addition of samples.

- **Stopping Reagents**

  Stopping reagents are added to prevent further enzyme reaction in ELISA. The stopping is usually made at a time when the relationship among the enzyme-substrate product is in the linear phase. Molar concentration of strong acids or strong bases stops enzyme activity by quickly denaturing enzymes. Some stopping reagents are enzyme-specific. Sodium azide is a potent inhibitor of HRPO, whereas EDTA inhibits Alkaline phosphatase by the chelation of metal ion cofactors.
  Since addition of stopping agents may alter the absorption spectrum of the product, the absorption peak must be known.

- **Laboratory conditions**

  The laboratory should be devoid of any acid fumes.

References

- https://www.abcam.com
- https://www.sinobiological.com
- https://www.bio-rad
- https://www.thermofisher.com