Biomatik
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Frequent Questions for ELISA Kits

1. **How should I store my ELISA Kit?**
   Components in the kits either need to be stored cold (2-8°C) or frozen (-20°C). Different kits may have different storage conditions, please check the indicated storage conditions upon receiving.

2. **How do I prepare my reagents?**
   Finish preparing the reagents 10 minutes before use. Concentrate all reagents to the bottom of the tube through centrifugation when using for the first time. Use precise measuring tools to ensure the most accurate preparation (pipette, graduated cylinder etc.). NEVER assume the quantity by adding directly to the supplied vials. Often, the amount in the tube is larger than specified. Ensure all labwear is clean before beginning.

3. **How do I separate my ELISA plate?**
   The strips provided are movable and it is recommended that any strips (wells) you do not intend to use immediately stay in the dark between 2-8°C. Avoid exposing the whole plate unless you intended to use the whole plate immediately. As a side note, leakage from the aluminum foil bag is alright, this will not affect the quality of the plate.

4. **How do I add my samples or reagents?**
   You should add all your samples within a 5-minute span. The time between each sample addition should be uniform to ensure reaction consistency.

5. **How do I incubation my plates?**
   Ensure the proper use of new, clean plate sealers to prevent sample evaporation and contamination. Maintain a steady hand when moving the plate in order not to spill the liquid. Avoid excessive opening of the incubator door to keep the temperature a consistent 37℃.

6. **How do I wash the wells?**
   Use the same volume of buffer per well, a multi-channel pipette is ideal. If you choose to use an ELISA washer, ensure it is clean and contamination free. Tap plates upside down on clean paper towel to dry. For a detailed procedure, please refer to your manual.

7. **How do I read my ELISA plate?**
   Begin reading the plate 5 minutes after the stop solution has been added. This helps to avoid a miss reading due to a buildup of precipitate. Ensure the microplate reader has been set correctly and the filter is properly calibrated. Avoid mixing reagents from different lot numbers, using a cap for any other tube than the one it originated, or replacing any components of a kit with those of another.

8. **Can detection of reagent A, B and the reconstituted standard be used once or multiple times?**
   Dilution for reagent A and B is not necessary to be done all at once. It is recommended they be kept in their stock solution to maintain stability. Only prepare as much reagent as you intend to use for that immediate experiment. The reconstitution standard however cannot be kept as it degrades after storage at low concentrations. It is recommended to only use the reconstitution standard once.

9. **Can the standard curve be extended?**
   Results for outside of the standard curve are not supported. Only results within the standard curve are reproducible and therefore accurate and in accordance with the kit.

10. **Why must I dilute my samples?**
    If your values fall above the standard curve, you must dilute your samples to bring them within the kit's

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detection range.

11. **Why is there a low sensitivity or low absorbance values?**
    Is the target protein not expressed in sample used, or is there a low level of target protein expression in sample used? Ensure the expression profile of the target protein will express in your samples. If there is low level of expression, try increasing the amount of sample used. You may also need to switch to a more sensitive assay. Confirm you are using a positive control within the detection range for this assay.

12. **How can I check if my kit is working before I begin my experiment?**
    Pre-coated plate, standards, detection reagent A, and detection reagent B are core reagents in the kit. If you are concerned about the kit quality after transit, you can run one or both of the following tests. If you are looking to conserve reagents and only validate TMB and detection buffer B, try Test #1. If you wish to validate the whole kit, run Test #2 or both Test #1 and #2.

    Test #1: This test is used to validate the TMB substrate and detection B after transportation. Draw 100ul of TMB substrate and place in a clean EP tube, this should be colorless. Using a new pipette, add in 1ul of the detection reagent B. Mix well and observe for color. If there is color present, it means that the TMB substrate and detection B are good - this is a good indicator that the whole kit is good after transit.

    Test #2: This test is used to validate the whole kit (validate all reagents). Run a pilot experiment with different dilutions of the standard with one or two samples to test. The standard curve can be used to determine whether the whole kit is still good or not, and it can also be served as the positive control and negative control both.

Contributing Factors for ELISA Failure

Many factors contribute to ELISA failure. Most of these results can be avoided by carefully reading and understanding the manual in its entirety before experiment. Please see the suggestions below for troubleshooting failed ELISA test:

- Check the kit's expiration date to ensure the test was carried out prior to expiry. Also ensure that all the reagents were stored properly, in accordance with the manual.
- Look for signs of deterioration in reagent solutions or instability. Some indications are precipitation or discoloration. Also ensure the substrate reagent is colorless.
- It is recommended that you use disposable plastic pipettes, tips, and containers to prepare and store the reagent. This helps to minimize cross contamination. It is also recommended to change pipette tips after every use.
- Ensure the manual indicated incubation time and temperature was achieved.
- Ensure the correct kit reagents were used and not substituted from a different kit.
- Finally, to improve accuracy, samples and standards should be run in duplicates.

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Common Problems

1. Incomplete washing: This can contribute to a poor standard curve or poor precision. Ensure the apparatus was soaked for an adequate amount of time.
2. Inadequate aspiration: This can contribute poor standard curve, poor precision, or too much signal. Ensure wells are completely dry after aspiration.
3. Pipetting error: This can contribute a poor standard curve, poor precision, or too much signal. Recalibrate the pipette if needed. Furthermore, consider setting duplicates for each assay.
4. Incomplete washing: This can contribute to poor precision, a poor standard curve, a high background, too much signal, or poor duplication. If the apparatus was not washed properly this can occur. Ensure the apparatus was soaked for an adequate amount of time and the wash solution is not contaminated.
5. Improper dilution of Standard: This can contribute to a poor standard curve. Ensure the diluent provided in the kit is used as a blank value, ensure the highest standard was diluted accurately, and ensure the 2- fold dilution series was completed accurately.
6. Unequal equal mixing of reagents: This can contribute to poor precision, or too much signal.
7. Unequal volumes: This can contribute to a poor standard curve. Ensure equal volumes are added to each well. If automatic pipetting, check pipette calibration.
8. Lack of HRP-Conjugate: this can contribute to poor discrimination against the standard curve. Ensure the correct dilution for the experiment.
9. Plate incubation time: Short incubation period can contribute to poor discrimination against the standard curve, or a low reading across the entire plate. Ensure the plate is incubated according to the kit or increase the incubation time.
10. Re-used plate seal: This can contribute to poor duplication. Ensure a new plate seal is used for each step of the procedure.
11. Lack of plate seal: This can contribute to poor duplication. Ensure a plate seal is used for each step of the procedure.
12. Contamination of the buffer: This can contribute to poor duplication. If you suspect the buffer is contaminated, prepare a fresh buffer.
13. Sample is too concentrated: This can contribute to a normal standard curve but sample OD is too high. Dilute samples and run the test again.
14. Incorrect wavelength: This can contribute to a low reading across the entire plate. Ensure to check both the filter and the reader to determine the cause.
15. Neglecting to add stop solution: This can contribute to a low reading across the entire plate. Ensure to add stop solution to each well.
16. Variation in incubation temperature: This can contribute to a low reading across the entire plate. Check the incubator to ensure even temperature.
17. Lost activity: This kit is not functioning properly. Check expiration date. Try a new kit.

Example Citations:

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