Bacterial Reagent Set

Indirect ELISA, Alkaline phosphatase label

List of contents

Lot number	Item	96 wells	500 wells	1000 wells	5000 wells
_____	Detection antibody	0.150 ml	0.275 ml	0.525 ml	2.525 ml
_____	Alkaline phosphatase enzyme conjugate	0.150 ml	0.275 ml	0.525 ml	2.525 ml
	The above items should be stored at 4° C.				
_____	96-well microtiter plates, strip or solid	1 strip	5 solid	10 solid	50 solid
	The above items can be stored at room temperature.				

Materials required, but not provided

- Carbonate Coating Buffer (formulation on page 5)
- PBST Wash Buffer (formulation on page 5)
- PBS Buffer (formulation on page 5)
- Nonfat Dry Milk (ACC 00420)
- PNP Substrate Buffer (formulation on page 5)
- PNP Substrate Tablets (ACC 00404)
- Buffer packs containing the above required items can be purchased from Agdia (ACC 00112)
- Distilled or purified water
- Knife or razor blade
- Sterile toothpicks
- Paper towels
- Micropipette
- Micropipette tips
- Airtight container for incubations

Limitations

Expiration: This test should be used within 1 year of purchase.

Storage: Test results may be weak or the test may fail if storage instructions are not followed properly.

Buffers: Do not store 1X buffers from day to day.

Dilutions: Read all labels carefully prior to preparing solutions to assure proper antibody concentrations. All antibody dilutions have been optimized for the greatest possible sensitivity and specificity based on available isolates and hosts. Using dilutions other than those listed can lead to potential false positives or false negatives.

Precautions

Prevent direct skin and eye contact with, or ingestion of, product components. Obtain medical attention in case of accidental ingestion of kit components. Always wash hands thoroughly after using this product.

Technical Service

If you have any questions about using this kit, please contact Agdia, Inc. Monday – Friday by phone (574-264-2014 or 800-622-4342) or by email (info@agdia.com).

Bacterial Reagent Set

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Preparing for the test

Prepare carbonate coating buffer

Prepare only as much 1X buffers that will be needed for one day. This buffer will be used for sample collection.

Sample preparation

When possible, select samples showing symptoms. Using a knife or razor blade, cut into sample vascular tissue to produce a bacterial stream. Place the cut end in 1 ml of water. The water can be centrifuged and the pellet resuspended in coating buffer; or a short serial dilution series of the water can be made in the ELISA well with coating buffer.

When testing bacterial cultures, a sterile toothpick can be used to collect the sample from a culture plate. Add collected sample to 1 ml of sample buffer and briefly agitate the toothpick. For more quantitative sampling, measure the solutions optical density at 600 nm and adjust to 0.01, which is equivalent to about 10^7 cfu/ml.

Test Procedure

1. Dispense samples

Following your loading diagram, dispense 100 µl of prepared sample into sample wells. Dispense 100 µl of positive control into positive control wells, and dispense 100 µl of 1X carbonate coating buffer into buffer wells.

2. Dry plate

Place the plate in an oven at 37° C overnight to dry. Be sure wells are completely dry before continuing. If not, replace in the oven until moisture is gone.

Prepare humid box

Prepare a humid box by lining an airtight container with a wet paper towel. Keeping testwells in a humid box during incubation will help prevent samples from evaporating.

3. Prepare blocking solution and block plate

Prepare blocking solution of 5% nonfat dry milk in PBS buffer (0.05 g nonfat dry milk per 1 ml PBS buffer). Add 200 µl of blocking solution to each well.

4. Incubate plate

Incubate the plate in a humid box for 30 minutes at room temperature.

5. Prepare detection antibody

Note: All antibodies and enzyme conjugates should be prepared in a container made of a material such as polyethylene or glass that does not readily bind antibodies. Do not use polystyrene, polypropylene or polycarbonate.

Note: 96 testwell reagents have a different dilution ratio than larger reagent sets. Please read label on detection antibody bottle for appropriate dilution ratio.

Note: Always prepare antibody within 10 minutes before use.

To prepare the detection antibody diluent, dilute concentrated PBST buffer with nonfat dry milk.

Example: If you will be preparing 10 ml of detection antibody diluent, first dispense 10 ml of PBST buffer, and then add 0.25 g of nonfat milk, mix thoroughly.

The detection antibody is provided in a concentrated solution and must be diluted with detection antibody diluent before use. The recommended antibody to buffer ratio is given on the label.

Prepare the volume of detection antibody diluent needed for the test. You will need 100 µl of detection antibody diluent for each test well you are using. A full plate will require about 10 ml. Then, add the appropriate volume of concentrated detection antibody to the detection antibody diluent at the dilution on the label.

Example 1: If the dilution given on the bottle of concentrated detection antibody is 1:200, and you are preparing 10 ml of detection antibody solution, you should mix 10 ml of detection antibody diluent with 50 µl of the concentrated detection antibody. Mix the prepared detection antibody solution thoroughly and use immediately.

Example 2: If the dilution given on the bottle of concentrated detection antibody is 1:100, and you are preparing 10 ml of detection antibody solution, you should mix 10 ml of detection antibody diluent with 100 µl of the concentrated detection antibody. Mix the prepared detection antibody solution thoroughly and use immediately.

6. Wash plate

When blocking incubation is complete, wash the plate. Use a quick flipping motion to dump the wells into a sink or waste container without mixing the contents.

Fill all the wells completely with 1X PBST, and then quickly empty them again. Repeat 2 times.

After washing, hold the frame upside down and tap firmly on a folded paper towel to remove all droplets of wash buffer.

7. Add detection antibody

Dispense 100 µl of prepared detection antibody per well.

8. Incubate plate

Set the plate inside the humid box and incubate for 1 hour at room temperature.

9. Prepare enzyme conjugate

Note: Always prepare enzyme conjugate within 10 minutes before use.

Note: 96 testwell reagents have a different dilution ratio than larger reagent sets. Please read label on enzyme conjugate bottle for appropriate dilution ratio.

To prepare the enzyme conjugate diluent, dilute concentrated PBST buffer with nonfat dry milk.

Example: If you will be preparing 10 ml of enzyme conjugate diluent, first dispense 10 ml of PBST buffer, and then add 0.25 g of nonfat milk, mix thoroughly.

The enzyme conjugate is provided in a concentrated solution and must be diluted with enzyme conjugate diluent before use. The recommended antibody to buffer ratio is given on the label.

Prepare the volume of enzyme conjugate diluent needed for the test. You will need 100 µl of enzyme conjugate diluent for each test well you are using. A full plate will require about 10 ml. Then, add the appropriate volume of concentrated enzyme conjugate to the enzyme conjugate diluent at the dilution on the label.

Example 1: If the dilution given on the bottle of concentrated enzyme conjugate is 1:200, and you are preparing 10 ml of enzyme conjugate solution, you should mix 10 ml of enzyme conjugate diluent with 50 µl of the concentrated enzyme conjugate. Mix the prepared enzyme conjugate solution thoroughly and use immediately.

Example 2: If the dilution given on the bottle of concentrated enzyme conjugate is 1:100, and you are preparing 10 ml of enzyme conjugate solution, you should mix 10 ml of enzyme conjugate diluent with 100 µl of the concentrated enzyme conjugate. Mix the prepared enzyme conjugate solution thoroughly and use immediately.

10. Wash plate

Empty the wells into a sink or waste container. Fill the testwells completely with 1X PBST, and then quickly empty them again. Repeat 7 more times.

Hold the plate upside down and tap firmly on a folded paper towel to remove excess liquid.

11. Add enzyme conjugate

Dispense 100 µl of prepared enzyme conjugate per well.

12. Incubate plate

Set the plate inside the humid box and incubate for 1 hour at room temperature.

13. Prepare PNP solution

Each PNP tablet (ACC 00404) will make 5 ml of PNP solution, at a concentration of 1 mg/ml, about enough for five 8-well strips.

About 15 minutes before the end of the above incubation step, measure 5 ml of room temperature 1X PNP buffer for each tablet you will be using. Then, without touching the tablets, add the PNP tablets to the buffer.

Note: Do not touch the PNP tablets or expose the PNP solution to strong light. Light or contamination could cause background color in negative wells.

14. Wash plate

Empty the wells into a sink or waste container. Fill the testwells completely with 1X PBST, and then quickly empty them again. Repeat 7 more times.

Hold the plate upside down and tap firmly on a folded paper towel to remove excess liquid.

15. Add PNP substrate

Dispense 100 µl of PNP substrate into each testwell.

16. Incubate plate

Incubate the plate for 60 minutes.

17. Evaluate results

Examine the wells by eye, or measure on a plate reader at 405 nm. Air bubbles which are present at the time of reading can alter results, if in the light path. Agdia recommends that bubbles be eliminated prior to reading.

Wells in which color develops indicate positive results. Wells in which there is no significant color development indicate negative result. Test results are valid only if positive control wells give a positive result and buffer wells remain colorless.

Results may be interpreted after more than 60 minutes of incubation as long as negative wells remain virtually clear.

Buffer Formulations

Carbonate Coating Buffer (1X)

Dissolve in distilled water to 1000 ml:

Sodium carbonate (anhydrous)	1.59 g
Sodium bicarbonate	2.93 g
Sodium azide	0.2 g

Adjust pH to 9.6. Store at 4° C.

PBS Buffer (1X)

Dissolve the following ingredients in 930 ml distilled water in the order listed. Add sodium phosphate slowly.

Sodium phosphate, dibasic (anhydrous)	1.15 g
Potassium chloride	0.2 g
Potassium phosphate, monobasic (anhydrous)	0.2 g
Sodium chloride	8.0 g
Sodium azide	0.2 g

Adjust pH to 7.4. Adjust final volume to 1000 ml with distilled water.

PBST Buffer (Wash Buffer) (1X)

Dissolve in distilled water to 1000 ml:

Sodium chloride	8.0 g
Sodium phosphate, dibasic (anhydrous)	1.15 g
Potassium phosphate, monobasic (anhydrous)	0.2 g
Potassium chloride	0.2 g
Tween-20	0.5 g

Adjust pH to 7.4

PNP Buffer (1X)

Dissolve in 800 ml distilled water:

Magnesium chloride hexahydrate	0.1 g
Sodium azide	0.2 g
Diethanolamine	97.0 ml

Adjust pH to 9.8 with hydrochloric acid. Adjust final volume to 1000 ml with distilled water. Store at 4° C.

Date _____ Test _____

Test performed by _____

	1	2	3	4	5	6	7	8	9	10	11	12
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B												
C												
D												
E												
F												
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