primediagnostics

Technical guidelines:

(V24-03)

Attention!

Preparation of MWS and SAsol is different for this kit

To obtain reliable and reproducible results, the operator should carefully read this entire manual before running the assay.

- Do not use the contents of the kit beyond expire date.
- Do not mix or substitute reagents with those from other lots or sources.
- The microspheres are light sensitive and must always be protected from the light. Cover the assay plate containing beads with aluminium foil during all incubation steps.
- Allow all reagents to warm to room temperature before use in the assay.
- All washings must be performed with the Wash Buffer provided.
- If the plate cannot be processed immediately it is possible to pause the assay at two different stages. After the bead incubation or the after secondary antibody incubation.
- To pause the assay; place the plate on the magnetic stand and wait for at least 1 minute to allow the beads to settle. Remove supernatant from the wells (flick plate; microtiter plate should be left on the magnetic stand!). Remove the plate from the magnetic stand. Add 100 µl of washbuffer to each well, cover the plate with a lid and aluminium foil and store the plate at 2-8°C for up to 24 hours.
- To resume the assay; Take the plate from the fridge, place the plate on the magnetic stand and wait for at least 1 minute to allow the beads to settle. Remove supernatant from the wells (flick plate; microtiter plate should be left on the magnetic stand!) and continue following protocol.
- Generally, storage after bead- or secondary antibody mixture incubation will not influence the kit performance. Storage of the plate during, or after, SA-PE incubation will influence the kit performance and is not advised, this step should be performed within 6 hours.
- The plate shaker should be set at a speed to provide maximum orbital mixing without splashing of liquid outside the wells.
- Mix all reagents well before adding to the plate.

The positive control included in this kit consists of inactivated virus. This control is intended to verify the Luminex performance and optimized for use in Luminex only. The control is not suitable for other applications like inoculation of plants. The positive control may contain more than one pathogen and may give a positive reaction with other target reagents as well. The antigenic reactivity of the positive control may, even under optimal storage conditions, decrease over time. However, the control can be used until the expiry date marked on the vial.

This kit can be used for the testing of plant material.
We strongly recommend using negative controls existing of extraction buffer only AND healthy plant extracts from the appropriate host of the pathogen in every test.
The positive control produced by Prime Diagnostics is a qualitative control and can be used as an internal control for the assay only. We recommend using an in-house positive control as well.

The working of this test is only guaranteed if the protocol is followed strictly. Any deviation from the protocol included in the kit (e.g., dilutions, volumes, times and/or temperatures) may cause loss of reactivity, specificity, selectivity and detection limits.

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Buffers & Controls

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PBS-Tween [10x]

Name	Concentration	Amount
PBS-Tween tablet(s)		
Adjust to volume, mentioned on bottle (A) with distilled water.		

Bead **W**orking **B**uffer [2x]

Name	Concentration	Amount
PBS-Tween [10x] (bottle A)	2x	1 mL
PRI Blocking agent (bottle B)	20 %	1 mL
Make up to 5 mL with distilled water (can be stored for 1 week at 4°C).		

Wash Buffer

Name	Concentration	Amount
PBS-Tween [10x] (bottle A)	1x	5 mL
PRI Blocking agent (bottle B)	5 %	2,5 mL
Make up to 50 mL with distilled water (can be stored for 1 week at 4°C).		

Controls:

Negative controls; add 50 µl of Healthy plant material or equivalent.

Positive controls; add 10 µl of PC + 40 µl of Healthy plant material or equivalent.

Up to three PC can be mixed into one well, provided that the total volume remains 50 µl. However, the following combinations should be avoided.

- INSV and TSWV
- CGMMV, ORSV, PMMoV, TMGMV, TMV, ToBRFV and ToMV
- PVM and PVS
- TNVb and TNVp

Protocol Manual washing

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1. This protocol is made for <u>one</u> 96-wells plate.

2. Prepare **Microsphere Working Solution (MWS)**:
   - Spin the **CMS** tubes (**1A** and **1B**) shortly before opening (max. 100_g for 5 seconds). Vortex the beads (**CMS** tubes **1A** and **1B**) vigorously for 2x 5 seconds. Spin **CMS** tubes **1A** and **1B** shortly before opening (max 100_g for 5 seconds). Mix the content by pipetting up and down five (5) times, using different pipet tips.
   - Transfer 50 µL of **CMS 1A** and 50 µL **CMS 1B** to 4.9 mL BWB [2x] and mix by vortex.
   - **Attention:** Do not spin after last mixing!

3. Add 50 µL Microsphere Working Solution (MWS) to all wells needed. (Samples + neg. control + pos. control(s)).

4. Next, transfer 50 µL sample per each well, as well as the pos. and neg. controls.

5. Cover microtiter plate with aluminium foil (to avoid photo bleaching).

6. Shake for 20 minutes (use appropriate shaker) and avoid sample cross over.

7. Prepare **S**econdary **A**ntibody **Sol**ution (**SAsol**):
   - Spin **SAS** tube (**2A** and **2B**) shortly before opening.
   - Transfer 50 µL **SAS 2A** and 50 µL **SAS 2B** to 9.9 mL Wash buffer (for each well you need 100 µL fresh prepared Secondary Antibody Solution).

8. Remove aluminium foil.

9. Wash microtiter plate:
   - Place microtiter plate on the magnetic stand.
   - Wait for at least 1 minute to allow the beads to settle.
   - Remove supernatant from the wells (flick plate; microtiter plate should be left on the magnetic stand!)
   - Add 100 µL Wash Buffer to each well (microtiter plate should be left on the magnetic stand!).
   - Wait 30 seconds.
   - Remove Wash Buffer from the wells (flick plate; microtiter plate should be left on the magnetic stand!).

10. If the sample contains a large amount of debris the washing can be repeated.

11. Remove the plate from the magnetic stand.

12. Add 100 µL **S**econdary **A**ntibody **Sol**ution (**SAsol** prepared in step 7) to each well.

13. Cover microtiter plate with aluminium foil.

14. Shake for 30 minutes (use appropriate shaker) and avoid sample cross over.

15. During the last 10 minutes of this incubation prepare fresh SA-PE solution. Spin **SA-PE** tube (**3**) shortly before opening. Add 1 µL SA-PE to 1 mL Wash Buffer. You need 75 µL of fresh prepared SA-PE solution per well. Keep tube in dark.

16. Remove aluminium foil.

17. Place microtiter plate on the magnetic stand.

18. Wait for at least 1 minute to allow the beads to settle.

19. Remove supernatant from the wells (flick plate; microtiter plate should be left on the magnetic stand!).

20. Add 100 µL Wash Buffer to each well (microtiter plate should be left on the magnetic stand!).

21. Wait 30 seconds.

22. Remove Wash Buffer from the wells (flick plate; microtiter plate should be left on the magnetic stand!).

23. Remove the plate from the magnetic stand. Add 75 µL of the SA-PE solution made in step 15.

24. Cover the plate with aluminium foil.

25. Shake for 15 minutes (use appropriate shaker) and avoid sample cross over

26. Place microtiter plate on the magnetic stand.

27. Wait for at least 1 minute to allow the beads to settle.

28. Remove supernatant from the wells (flick plate; microtiter plate should be left on the magnetic stand!).

29. Add 100 µL Wash Buffer to each well.

30. Shake for 5 minutes (avoid cross over)

31. Analyse samples

PRI Blocker

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Long term storage

For long term storage PRI Blocker should be stored at -20°C upon arrival and is stable for 1 year.

Using PRI Blocker

Before usage PRI Blocker should be thawed at room temperature or refrigerator. Don't use microwave or heated water bath. Once thawed PRI Blocker should not be frozen again.

After thawing, PRI Blocker is stable for 1 month, and should be kept on ice during usage and stored at 4°C afterwards.

Lyophilized positive Controls

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Contents and storing

A lyophilized positive control consists of vials containing lyophilized leaf material. The unprepared lyophilized positive control can be stored at 4°C for 1 year.

Preparing positive controls

All lyophilized positive controls except CMV are dissolved by adding 120 µl of demineralized water to each vial containing lyophilized positive control. For the lyophilized positive control **CMV** add **180 µl**. Leave the vial at room temperature for circa 5 minutes, then mix the contents by shaking gently. Lyophilized positive controls are provided in a vial large enough to contain the prepared liquid. The positive control is now ready for use.

Storing and using the prepared positive controls

The lyophilized positive control can be used with Luminex xMAP assays like glycerol positive controls. Add 10 µl positive control to a well and fill up a of volume of 50 µl. this must be done at the same time as the sample extracts.

After preparing the lyophilized positive control, divide it into aliquots of 24 µl. Each aliquot is sufficient for 2 wells plus a small additional volume to assure easy dispensing.

Aliquots of prepared positive control must be stored frozen (-20°C freezer or household freezer). Do not thaw until just before use and freeze immediately after the first-time use.

Prepared lyophilized positive control can be stored at -20°C for a maximum of 6 months.

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