PCR Test Kit

ACDamp Immunocapture Real-Time RT-PCR Kit

Lot number	Item	24 Tests	48 Tests	Storage
	1X8 PCR strips coated with capturing antibody	3 strips	6 strips	2-6°C
	Real-Time RT-PCR primers and probes, freezing-dried	1 vial	1 vial	2-6°C
	PCR Sample Buffer, powder	2.3 g	4.6 g	2-6°C
	Washing Buffer, powder	4.8 g	9.6 g	2-6°C
	Tween-20, for sample and washing buffers	1.2 g	2.5 g	2-6°C
	Positive Controls-virus, 1.0 ml/bottle	1 bottle	1 bottle	2-6°C
	Negative Control, 1.0 ml/bottle	1 bottle	1 bottle	2-6°C
	Optical cover	1	1	RT
	Instruction	1	1	-

The following materials are not included, but required:

-Pipette and pipette tips
-Distilled water or other purified water
-Humid incubation container
-Glass wares, plastic wares, paper towels or other lab supplies
-Enzymes, master mix and PCR facilities

Safety and Storage

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

All reagent components should be stored at the recommended temperature to assure their full shelf life. The kit should be used within six months of purchase.

Please contact AC Diagnostics, Inc. if you have any questions about safety and storage of this product.

Preparing For the Test

Make sure all laboratory equipments and facilities required are ready for the test. Prepare a humid box for incubation steps

Kit Components

Check all the components are present in the package of PCR Kit by referring to the Content List. Familiarize yourself with the listed components and read this instruction before starting the test.

Prepare Buffers from Powders

To prepare the 1x buffers, dissolve the buffer powder into D.H_2O and add tween-20 at the ratios on the table below. For sample buffer, mix the powder with small amount of D.H_2O into a paste (no clumps) before adding more D.H_2O. Stir for 10-30 minutes for dissolving completely and make up to final volume. Prepared 1x working solution can be stored at refrigerator (2-6°C) for up to 3 months. If you have any questions about preparing and using the buffers, please contact AC Diagnostics.

	1x Sample buffer		1x Washing buffer	
Buffer Powder	2.3 g	4.6 g	4.8 g	9.6 g
Tween-20	1.0 g	2.0 g	0.2 g	0.5 g
Final Volume	100 ml	200 ml	500 ml	1000 ml

Prepare Controls

Add 1.0 ml of sample extraction buffer into the bottles of lyophilized positive and negative controls, and mix by gently inverting the bottles until fully dissolved.

The prepared control can be used immediately, or divided into aliquots and stored frozen (-10 to - 40°C). Each aliquot should be sufficient for at least one use. For example, if you will use this control in one well each time you run the test, prepare 60 µl aliquots. Prepare 120 µl aliquots if you will use the control in two wells.

Control aliquots must be kept frozen until just before use. Do not refreeze controls once they have been thawed. Using the Control at the time you run the test, remove one control from storage and allow it to thaw. Add 50 µl of the prepared control to the appropriate control well.

Prepare PCR Tubes

Warm the PCR tube package to room temperature before opening. Remove the PCR strips from foil Pouch, seal the rests in the pouch with the desiccant, and store at 2-6°C. Put the strips in a PCR tube rack and mark the strips in case a strip becomes separate from the rack.

Make a copy of the attached recording sheet and create a loading diagram by recording the locations of your samples, controls, and other reagents needed.

Prepare Samples

Select symptomatic and/or infective tissues for the test. Leaf tissue is often used in ELISA testing. How ever, plant tissues such as stem, sprout, seed, tuber, root and others can also be tested.

Single sample is suggested to be used in each test well. In some cases, composites of up to ten leaves per test well can be used to make testing more economical. However, too many plant samples per well can reduce the sensitivity of the test.

ACD's PCR sample buffer is used as extraction buffer. Grind sample with a mortar and pestle, or other grinding devices such as German Grinder or sample bag. If you are using a mortar and pestle, wash and rinse it thoroughly between samples.

If you extract plant sap, dilute the sap into sample extraction buffer at a ratio of 1:10 (sap volume: buffer volume). Or you can grind plant tissue in extraction Buffer at a 1:10 ratio (tissue weight: extraction Buffer volume).

If you have any questions about sampling and sample preparation, please contact AC Diagnostics, Inc.

Prepare Primers and Probes

The primers and probes in the vial are in lyophilized form. Reconstitute the primer by adding nuclease-free water in a volume labeled on the vial. Briefly spin down the contents in the vial, add the water into the vial and mix well by gently vortexing before use.

The prepared primer solution can be used immediately, or divided into aliquots and stored frozen (-10 to -40°C). Each aliquot should be sufficient for one PCR assay depending on the number of samples to be tested.

Prepare Real-Time RT-PCR Reaction Mixture

Specific PCR primers are include in this kit. It is suggested that RT-PCR reaction mixture is prepared basing on your Lab protocol. The recipes in following table are presented for your reference. Prepare the reaction mixture immediately before use.

Reagent	25µl/ Reaction
Nuclease-free Water	18.25 µl
10X Taq Polymerase Buffer	2.50 µl
Magnesium Chlorite, 25mM	2.00 µl
dNTPs (2.5 mM each)	0.80 µl
AMV Reverse Transcriptase (10 U/µl)	0.10 µl
Taq polymerase (5 U/µl)	0.20 µl
Rnasin® Plus RNase Inhibitor (40 U/µl)	0.15 µl
Primers and Probes, 10 µM each of F&R, and probe	1.00 µl

Immunocapture Real-Time RT-PCR Procedure

Sample Dispensing

Following your loading diagram on your recording sheet, dispense 50 µl of prepared sample into sample tubes. Dispense 50 µl of positive control into positive control tubes, and 50 µl of negative control into negative control tubes.

PCR Plate Incubation

Put the plate inside the humid box and incubate for 2-3 hours at room temperature (21-24 °C) or overnight in the refrigerator (4° C).

Washing PCR Plate

Wash the plate when the incubation is complete. Push the PCR strip tubes down to the rack and make sure it will not separate from the rack during washing. Hold the rack and use a quick flipping motion to empty the wells into a sink or waste container without mixing the contents among tubes.

Wash the plate by immediately filling the tubes with washing buffer, then quickly emptying them again. Repeat 6 to 8 times with the washing buffer. Then, rinse one time with distilled water. To remove trace drop of water from the tubes after washing, hold the rack upside down and tap firmly on a folded paper towel for several times.

Thorough and careful washing is very important for removing all plant inhibitors from the tube. Make sure to completely empty the tube for each washing, and to avoid cross-contamination among the tubes.

Real-Time RT-PCR Reaction mixture Dispensing

Dispense 25 - 50 µl of prepared PCR reaction mixture per tube to all of the tubes of each assay. Seal all tubes with optical cover.

Real-Time RT-PCR Cycling

PCR cycling reaction is conducted following instruction of the Real-Time RT-PCR machine used at your lab. Suggested cycling profile is in following table:

Cycle	Temperature	Time
1	55 °C	10 minutes
1	95 °C	2minutes
40	95°C	15 Second
	60°C	30 Second

Evaluation

Real-Time PCR amplification product is analyzed by fluorescence signal. Assay results of the samples are evaluated by comparing with the results of positive and negative controls.

Test results are valid only if positive control tubes give a positive result and negative control tubes remain negative.

RECORDING SHEET FOR Real-Time PCR ASSAY

TEST: _____ **DATE:** _____ **BY:** _____

TIMING: Sample Incubation: _____ **PCR Reaction:** _____ **C.V. Value :** _____

KEY POINTS: _____

REAGENTS: Sample buffer: _____ **Washing Buffer:** _____

Primers: _____ **Master Mix:** _____

Enzymes: _____ **Others:** _____

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

RESULTS/CONCLUSIONS:

1. _____
2. _____
3. _____