

User Manual

SignalChem Diagnostics

Aggressive ***Neopestalotiopsis* spp.** **(Neo-P) One-Pot Detection Kit**

To enable point-of-care testing for the immediate detection of aggressive Neo-P in plant samples

Catalog # AN001-K398 (Starter Kit)
Catalog # AN002-K398 (Standard Kit)

Diagnostic Reagents For Accurate And Reproducible Results

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Introduction

This testing kit is designed for the rapid detection of pathogens in plants. The kit is self-sufficient, so there is no need for the end user to send samples elsewhere for analysis. The kit is designed to work with an instrument (CRISPOT® Detection Device, Cat# CSP01-V19A, SOLD SEPARATELY), which screens for the presence of a portion of the pathogen's genome in all samples, thus identifying infected plants. It is important for the end-user to label the samples according to the plants they were extracted from to avoid confusion. The user is expected to spend around 5-10 minutes on sample preparation and setting up the device. Once set-up is complete, running the test on the detection device will take 25 minutes.

This kit specifically recognizes certain strains of unnamed "aggressive *Neopestalotiopsis* spp." (sometimes referred to as "*Neopestalotiopsis hispanica*") that were first characterized in Florida and North Carolina. Some additional *Neopestalotiopsis* strains or species that can also cause disease may not be detected by this kit. Please contact us if you are unsure whether this product will be useful for your application.

Disclosure

This product is not to be used as a drug, medical device, food additive, cosmetic, or household chemical. It is not to be used in therapeutic, consumer, or pesticidal applications. Not for resale.

Support

For inquiries and assistance, please contact us at:

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Version History

Version 4.0

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Kit Components

Starter Kit-Only Components

#	Component	Amount
1	Tube Rack	1
2	Adjustable Pipette	1

Not Provided

Gloves
Scissors or razor blades
*(not included with test kits,
available as a separate add-on)*

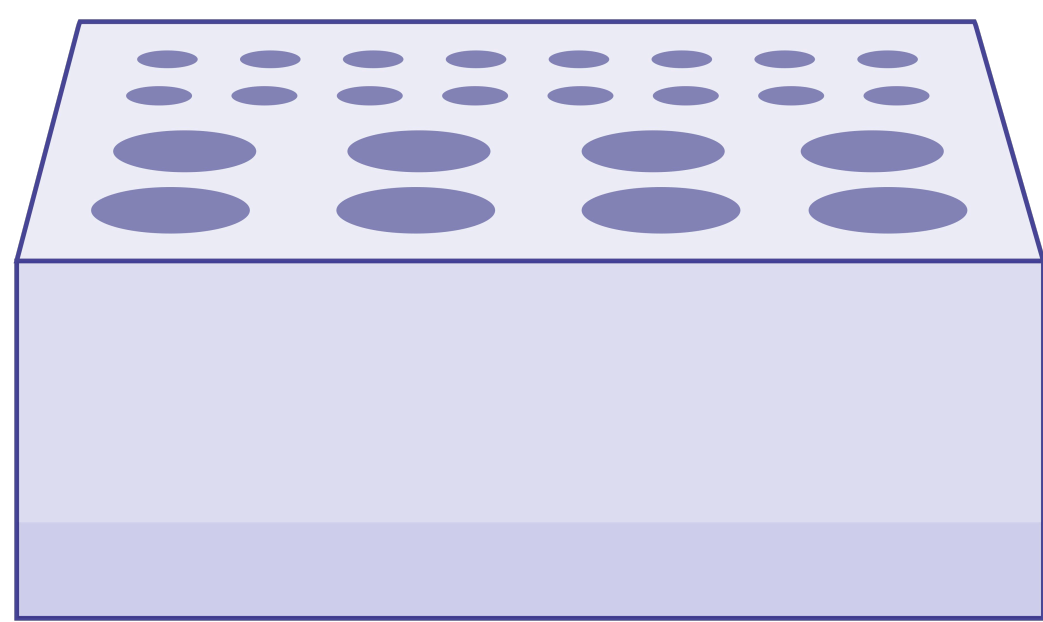
Standard Kit Components

#	Component	16 Reactions	32 Reactions	48 Reactions
3	Pipette Tips	50	100	150
4	Grommets	16	32	48
5	Pestles	16	32	48
6	1.5 mL Tubes With Lysis Buffer	16 tubes	32 tubes	48 tubes
7	1.5 mL Tubes With Reaction Buffer	16 tubes with red cap	32 tubes with red cap	48 tubes with red cap
8	PCR Tube Strips With Master Mix	2 (8-well) strip tubes	4 (8-well) strip tubes	6 (8-well) strip tubes
9	User Manual	1	1	1

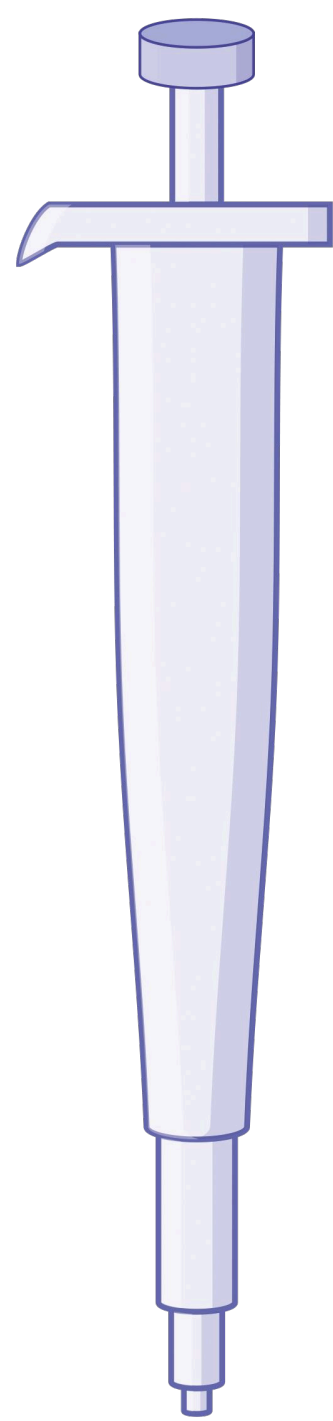
Kit Components: Visual Guide

Starter Kit-Only

1 Tube Rack

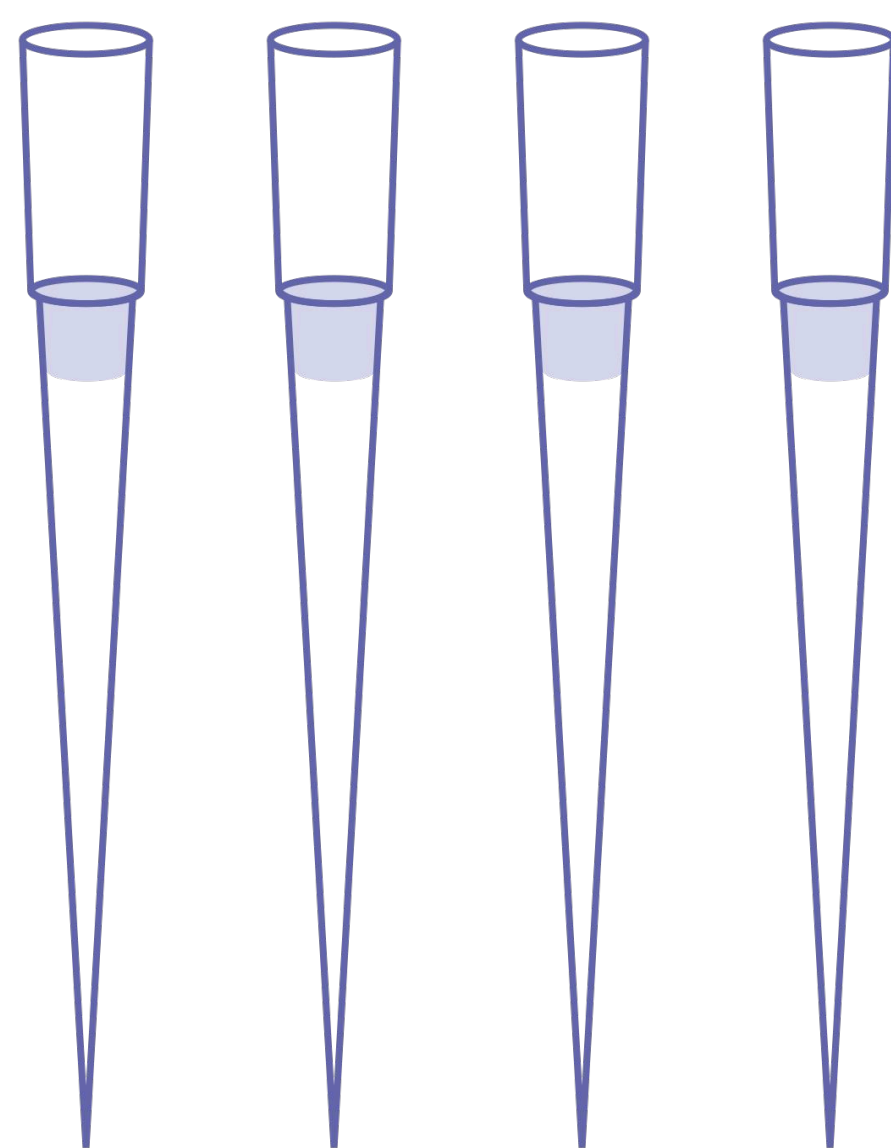


2 Adjustable Pipette

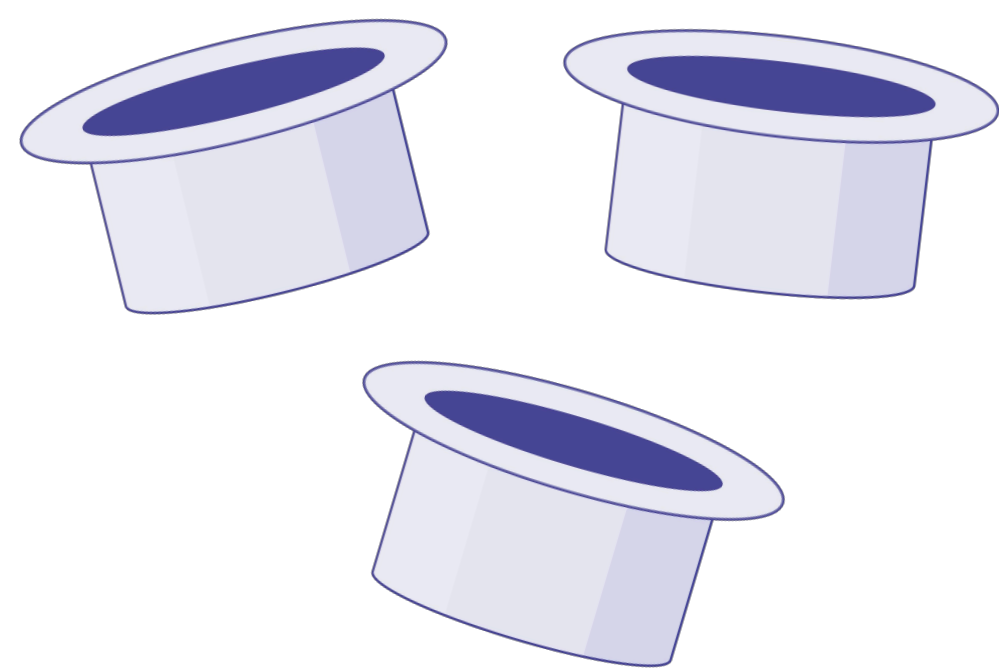


Standard Kit

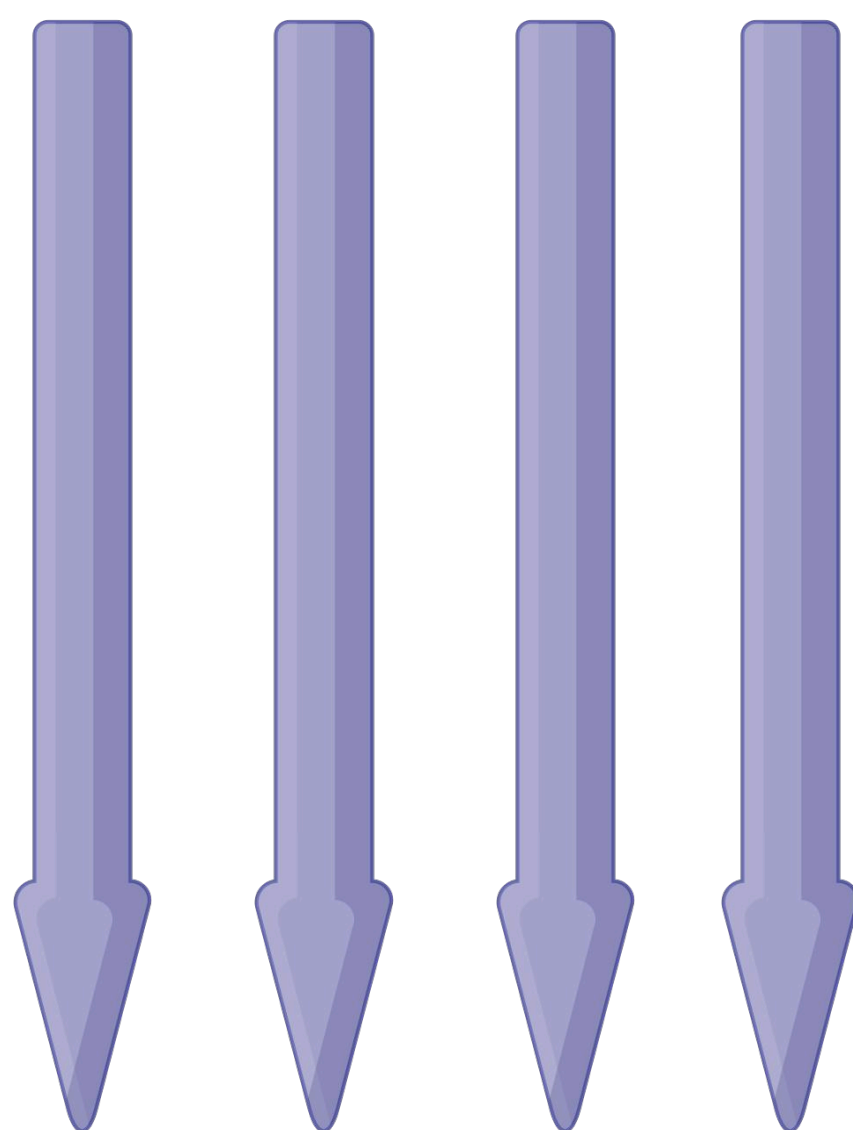
3 Pipette Tips



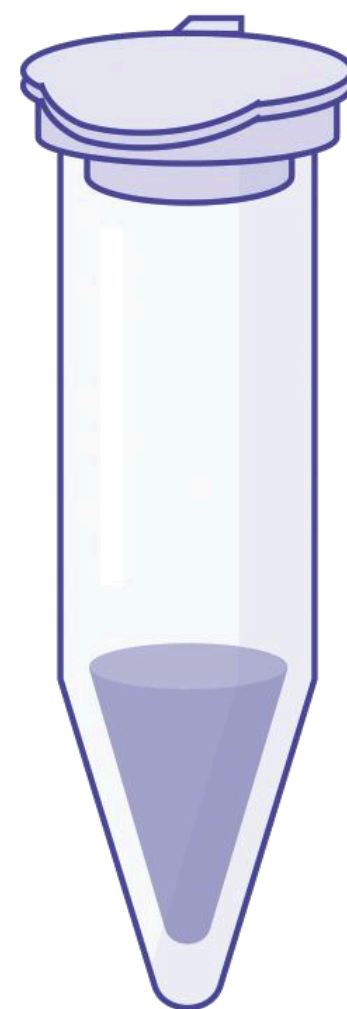
4 Grommets



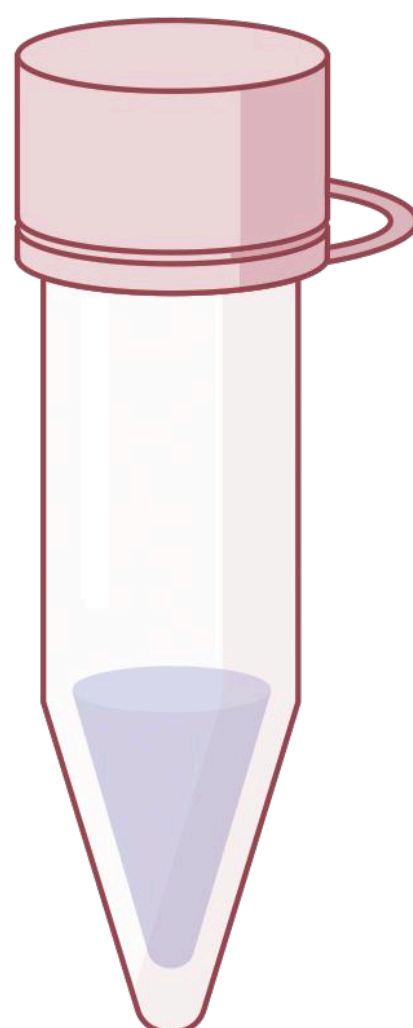
5 Pestles



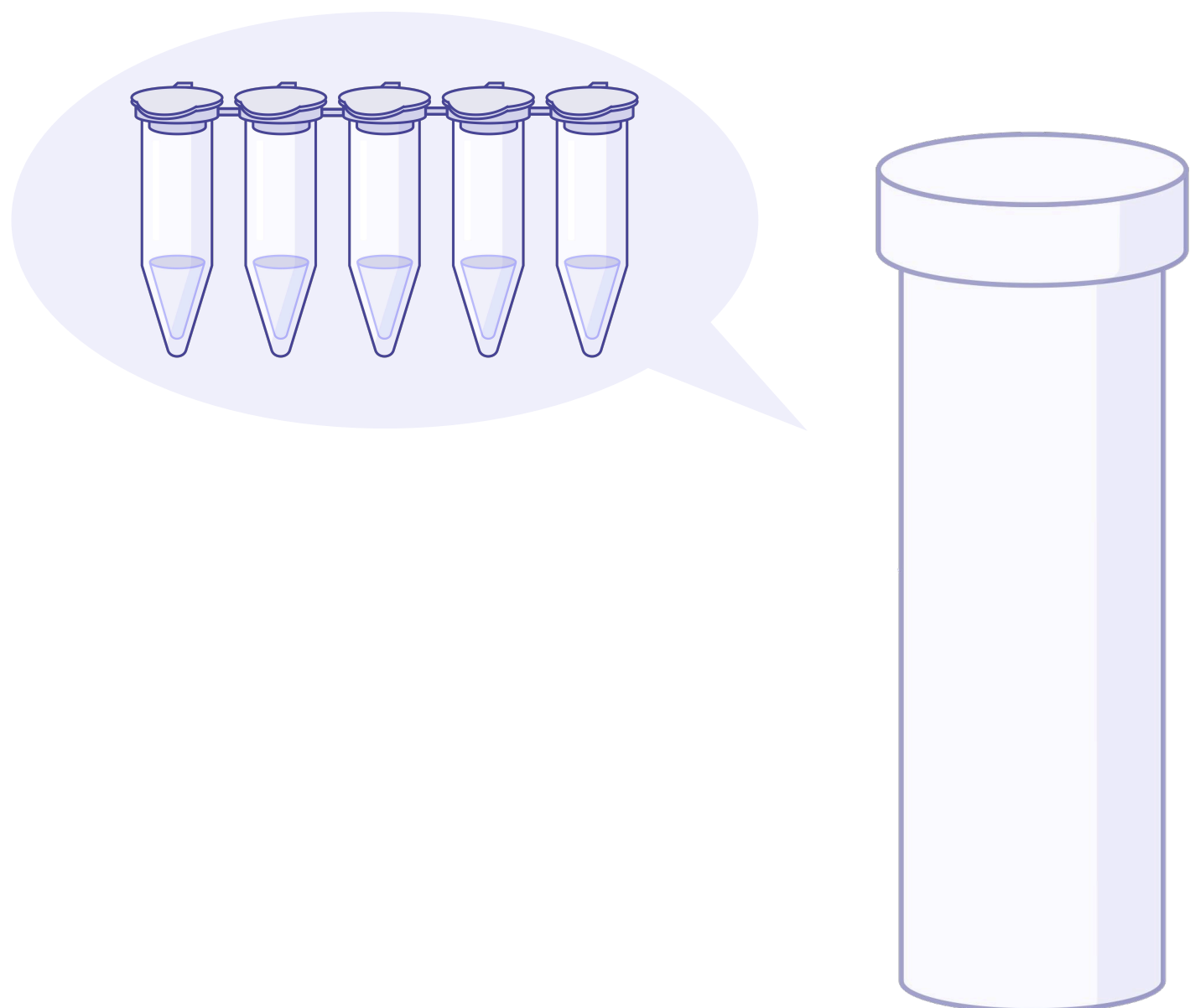
6 1.5 mL Tubes With Lysis Buffer



7 1.5 mL Tubes With Reaction Buffer



8 PCR Tube Strips With Master Mix in White Plastic Tube



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Storage and Precautions

Kit Storage

Store all kit components at 2-8°C for up to 12 months or at 8-25°C for up to 6 months.

The premixtures in the PCR tubes (clear tubes, with pellet) should be protected from light. The white storage tube or foil bag with desiccant offers light and moisture protection as long as the tube/bag is tightly closed. Always keep the strips in the tube/bag when not in use, as exposure to light or moisture can cause deterioration and lead to inaccurate results.

Precautions

- Ensure the working environment is clean before starting the experiment.
- Always wear a new pair of examination gloves when handling samples and performing the assay.
- Avoid cross-contamination between different samples
- The presence of inhibitors can lead to invalid or false-negative results.
- Mutations in the target regions of the Aggressive *Neopestalotiopsis* spp. genome, covered by the primers in this kit, may prevent the detection of the pathogen.
- Adhere to good laboratory practices to ensure proper use of this kit. Keep both the kit and reactions free from contamination. Carefully inspect all reagents for signs of contamination, and do not use any reagents that appear contaminated.
- Change gloves or decontaminate gloves and tools with 10% bleach between samples to avoid cross-contamination.
- Use new grommets between each sampling to avoid cross-contamination.
- Use a new pipette tip for each sample transfer to prevent cross-contamination.
- Dispose of used materials in the regular trash.

Sample Preparation: Quick Reference Guide

Protocol

These instructions are meant as a quick-start instructions for users familiar with the protocol. Please refer to the full visual guide for comprehensive instructions.

1. Prepare materials

- Determine the number of samples to test. Label each plant you are testing and record the sample order.
- For each sample, collect the required components from the kit (1 each for each sample):
 - Grommet
 - Pestle
 - Lysis Buffer Tube
 - Reaction Buffer Tube
 - Master Mix Tube
- Label lids of the tubes with the corresponding sample ID.

IMPORTANT: Ensure most of contents in tubes are at the bottom of the tube.

2. Collect samples

- Collect tissue according to instructions below. After tissue is collected, proceed to step 3 promptly. Do not allow intact or ground tissue to sit in lysis buffer for >10 minutes.
- For leaves:
 - Using a clean grommet, punch out tissue from areas suspected to be infected.
 - Ideally collect 4 leaf punches, but up to 8 can be used.
 - With a pipette tip (or pestle), eject tissue directly into lysis buffer (clear tube) and close the lid securely.
 - Repeat the steps above for all samples using a clean grommet and clean tip.
- For crown:
 - Cut the crown in half lengthwise (vertically) using a clean razor blade or clean scissors. Using the provided grommet, punch/core plant tissue from the cut surface in areas with suspected infection.
 - Push out the crown core from the grommet using a pipette tip (or pestle). Using a razor blade or scissors, cut cross sections of the core to get two 1 mm-thick discs. Place the pieces directly into the tube containing the lysis buffer. Close the lid securely.
 - Repeat the steps above for all samples using a clean razor blade, clean grommet and clean tip.
- For leaf stalks and pedicels:
 - Using a clean razor blade or scissors, cut 2 pieces of pedicels (each 1-2mm diameter by 5mm length), or cut 1-2 pieces of leaf stalk (each 2-3 mm diameter by 5 mm length).
 - Add tissue directly into lysis buffer (clear tube) and close securely.
 - Repeat the steps above for all samples using a clean blade/scissors.

Sample Preparation: Quick Reference Guide

Protocol (Continued)

- For fruit:
 - Using a clean razor blade or scissors, cut 1 pieces of fruit (1 cm x 0.5 cm x 0.5 cm). This should be around 200 mg.
 - Add tissue directly into lysis buffer (clear tube) and close securely.
 - Repeat the steps above for all samples using a clean blade/scissors.

3. Prepare samples and mix with reaction buffer

- Using a clean plastic pestle, grind and crush the tissue against the side/bottom of the tube until no large chunks remain (~30s - 1 min with moderate pressure).
- Wait ~30 s - 1 min to allow large debris to settle.
- Set the adjustable pipette to "5 µL" and attach a clean pipette tip.
- Transfer 5 µL of the extract to the reaction mixture tube (red-capped). Mix by pipetting up and down several times.
- Close the lid of the tube and invert or flick the tube to mix the contents.
- Tap the tube on a surface to ensure most of liquid is at the bottom.
- Repeat the steps above for all samples using new tips.
- The transfer/dilution should be done promptly after grinding. Do not allow intact or ground tissue to sit in lysis buffer for over 10 minutes.

4. Add samples to master mix

- Set the adjustable pipette to 30 µL and attach a new tip.
- Carefully open the lid for one diluted sample (red-capped). Transfer 30 µL of the diluted sample into a master mix tube without touching the pellet.
- Close the master mix tube, discard the tip. Close the diluted sample tube.
- Repeat the steps above for all samples using new tips each time.
- Mix the master mix tube(s) contents by flicking to fully resuspend the pellet.

CRITICAL: Check that all liquid is at the bottom of the tube and no large bubbles are present before proceeding to ensure accuracy.

5. Run test in CRISPOT®

- Preheat the device by running the heating program on the device for 1 minute until the temperature reaches 60°C.
- Place test strip (or portion if <8 samples) into CRISPOT® device.
- Select and start the Program 1 by pressing the bottom "play" button.

6. Read results

- After the run is completed, the infection status of each sample will be displayed: red for positive (detected) and green for negative (not detected).
- Record results immediately (write down or photograph), as they will be inaccessible if any buttons are pressed or the machine is turned off.

Sample Preparation: Quick Reference Guide

Protocol (Continued)

7. Dispose of materials

- DO NOT open master mix tubes once they have been tested in the CRISPOT®.
- All kit materials can be disposed of in regular garbage.
- Dispose of razors into sharps containers.

Using the Pipette

1. The pipettes are adjustable to between 5 µL and 50 µL. Turn the dial to adjust the volume.
2. Add a pipette tip to your pipette and ensure it is attached securely.
3. Press the plunger down to the first stop (where you first feel resistance).
4. Insert the pipette tip into the liquid.
5. Slowly release the plunger to draw up the desired volume. Observe the liquid enter the pipette tip. Ensure no air or debris enters the tip. Ensure no debris is blocking the tip hole.
6. Move the tip to the target container.
7. Press the plunger slowly but firmly to the 2nd stop (past the first point of resistance). Observe the liquid exit the pipette tip.
8. While keeping the plunger fully pressed, withdraw the tip from the liquid before releasing the plunger to avoid drawing any liquid back in.
9. Dispose of pipette tip by pressing the ejector.

Sample Preparation: Full Comprehensive Visual Guide

Comprehensive instructions for first-time users.

Protocol

1. Prepare materials

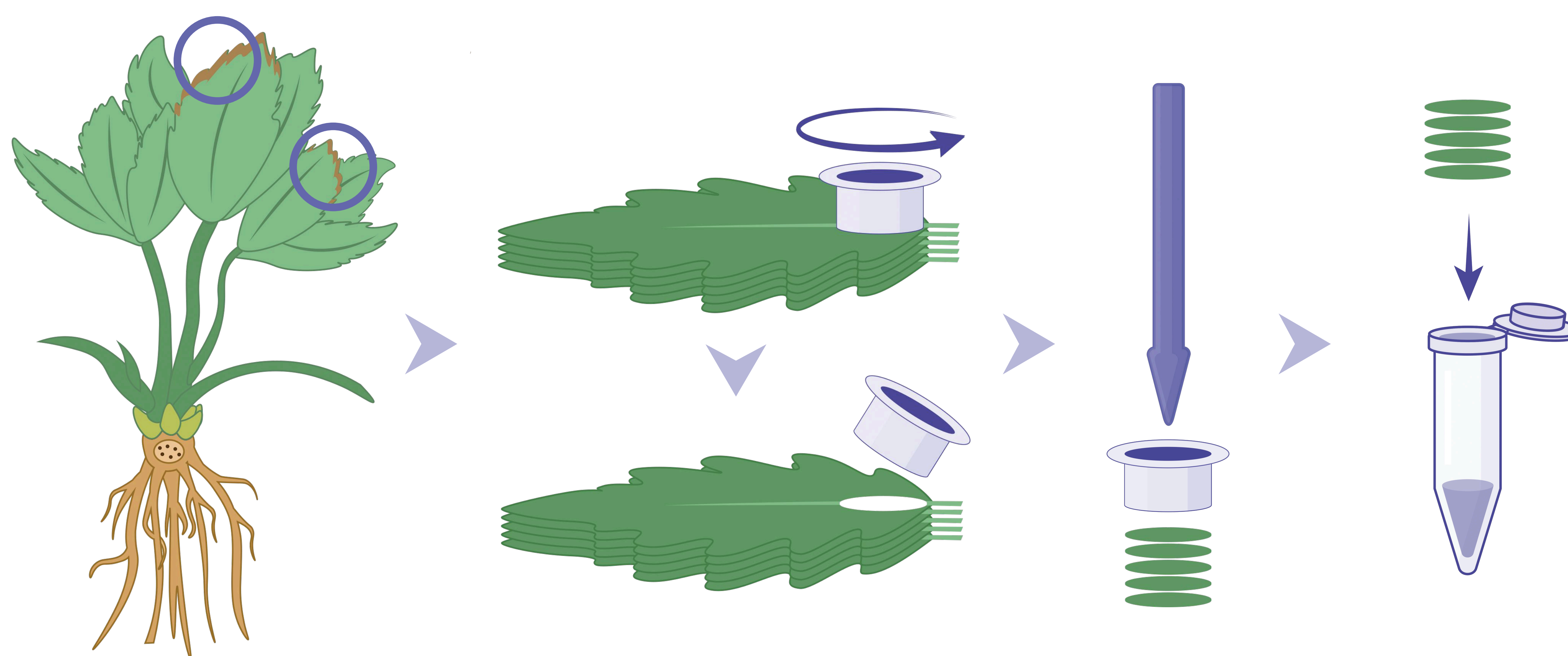
- Determine the number of samples to test. Label each plant you are testing and record the sample order. For each sample, collect the required components from the kit (1 each for each sample), including: grommets, pestles, Lysis Buffer Tube (Clear), Reaction Buffer tube (Red-capped), Master mix tube (PCR tube with dry pellet). If necessary, separate the needed number of tubes from the PCR tube strips. Label lids of the tubes with the corresponding sample ID. Ensure most of contents in tubes are at the bottom of the tube. If it's not, lightly tap the tubes on a flat surface or use a wrist flicking technique to get the contents to the bottom.

2. Collect samples

- Collect tissue according to instructions below. After tissue is collected, proceed to step 3 promptly. Do not allow intact or ground tissue to sit in lysis buffer for >10 minutes.

FOR LEAVES

- Using a clean grommet, punch out tissue from areas suspected to be infected. This can be done more easily with the leaf against a piece of paper towel or a hard surface. The margins of the suspected infection are good places to sample. Avoid brown/decayed tissue, excess soil and moisture. Ideally collect 4 leaf punches, but up to 8 can be used. To save time, punch all four punches and add them to the tube all at once. With a pipette tip (or pestle), eject tissue directly into lysis buffer (clear tube) and close the lid securely. Do not exceed recommended amounts. Repeat the steps above for all samples using a clean grommet and clean tip.

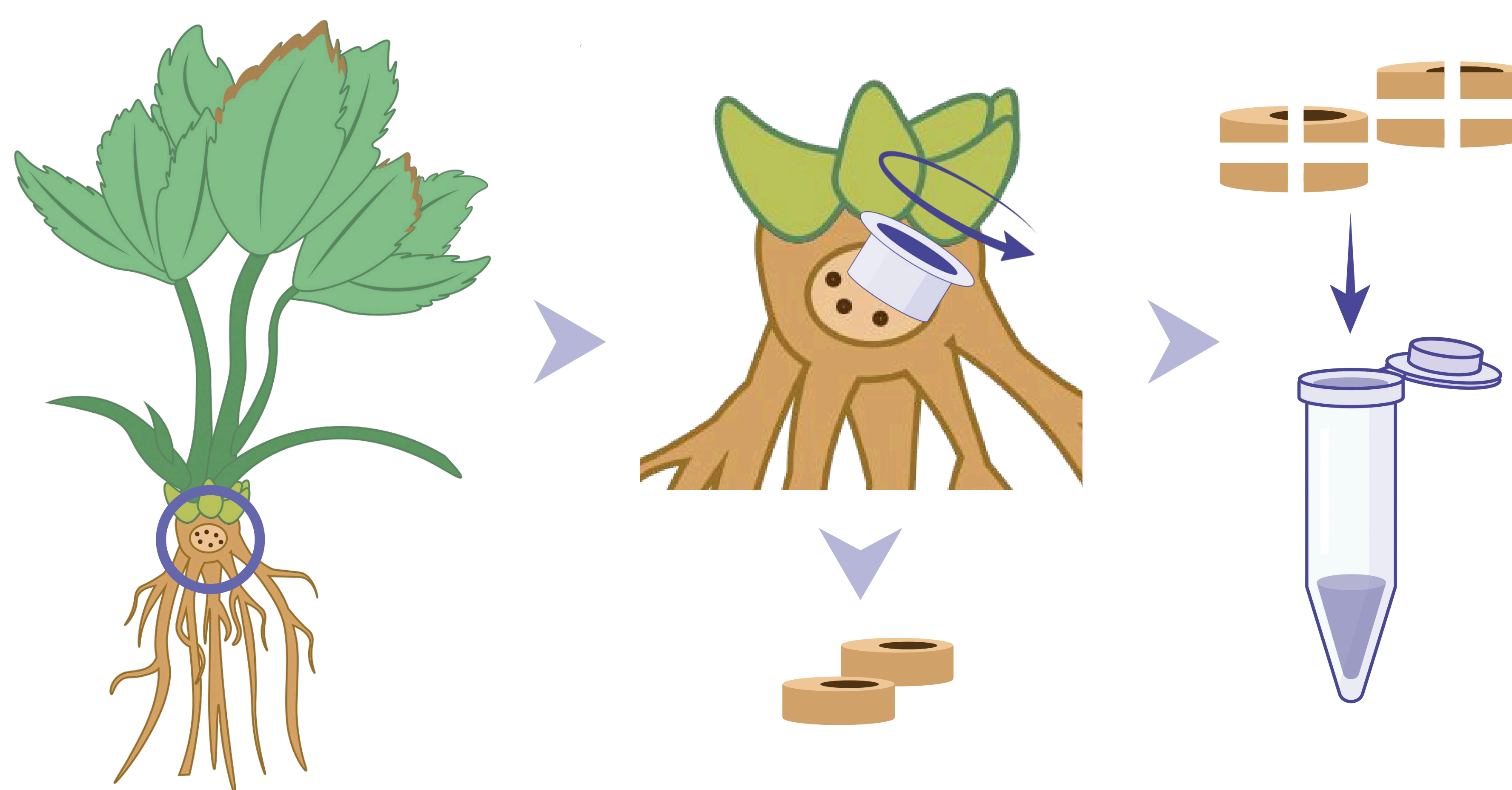


Sample Preparation: Full Comprehensive Visual Guide

Protocol (Continued)

FOR CROWN

- Cut the crown in half lengthwise (vertically) using a clean razor blade or clean scissors. Using the provided grommet, punch/core plant tissue from the cut surface in areas with suspected infection. Infected areas appear brown or orange compared to white/cream. Preferably collect from the fleshy pith rather than the cortex surrounding the crown. Avoid excessive soil or moisture. Push out the crown core from the grommet using a pipette tip (or pestle). Using a razor blade or scissors, cut cross sections of the core to get two 1 mm-thick discs. This should be ~30-40 mg total. The discs can be further cut to make grinding easier if desired. Place the pieces directly into the tube containing the lysis buffer. Close the lid securely. Repeat the steps above for all samples using a razor blade, clean grommet and clean tip.



FOR LEAF STALKS AND PEDICELS

- Using a razor blade or scissors, cut 2 pieces of pedicels (each 1-2 mm diameter by 5 mm length), or cut 1-2 pieces of leaf stalk (each 2-3 mm diameter by 5 mm length). Add tissue directly into lysis buffer (clear tube) and close securely. Repeat the steps above for all samples using a clean blade/scissors.

FOR FRUIT

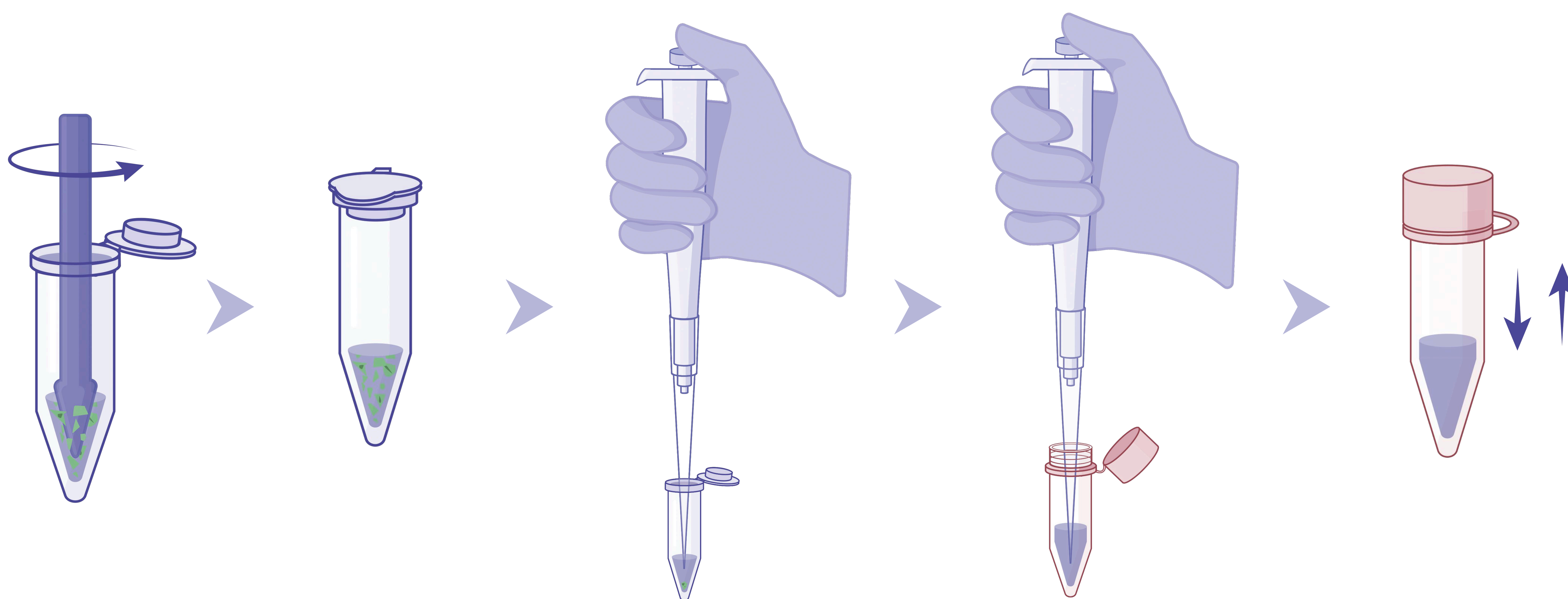
- Using a razor blade or scissors, cut 1 pieces of fruit (1 cm x 0.5 cm x 0.5 cm). This should be around 200 mg. Add tissue directly into lysis buffer (clear tube) and close securely. Repeat the steps above for all samples using a clean blade/scissors.

Sample Preparation: Full Comprehensive Visual Guide

Protocol (Continued)

3. Prepare samples and mix with reaction buffer

- Using a clean plastic pestle, grind and crush the tissue against the side/bottom of the tube until no large chunks remain (~30 s - 1 min with moderate pressure). Wait ~30 s - 1min to allow large debris to settle. Set the adjustable pipette to "5 μ L" and attach a clean pipette tip. Inserting the pipette tip just below the surface of the liquid to avoid debris, transfer 5 μ L of extract to the reaction mixture tube (red-capped). Mix by pipetting up and down several times. Close the lid of the tube. Invert or flick the tube to mix the contents. Tap the tube on a surface to ensure most of liquid is at the bottom. Repeat the steps above with all samples using new tips. The transfer/dilution should be done promptly after grinding. Do not allow intact or ground tissue to sit in lysis buffer for over 10 minutes. After dilution, the samples can be left while additional samples are processed.

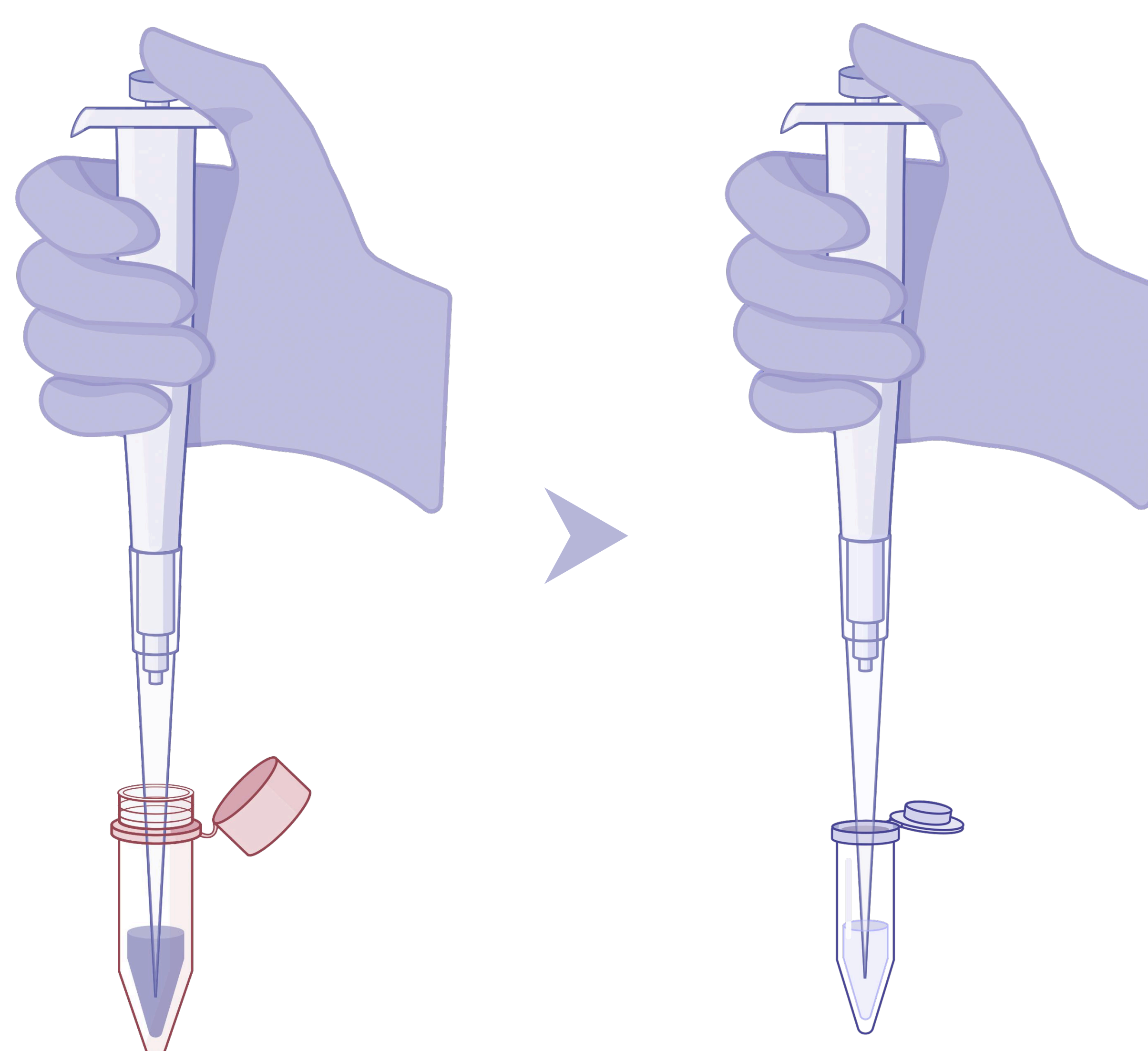


Sample Preparation: Full Comprehensive Visual Guide

Protocol (Continued)

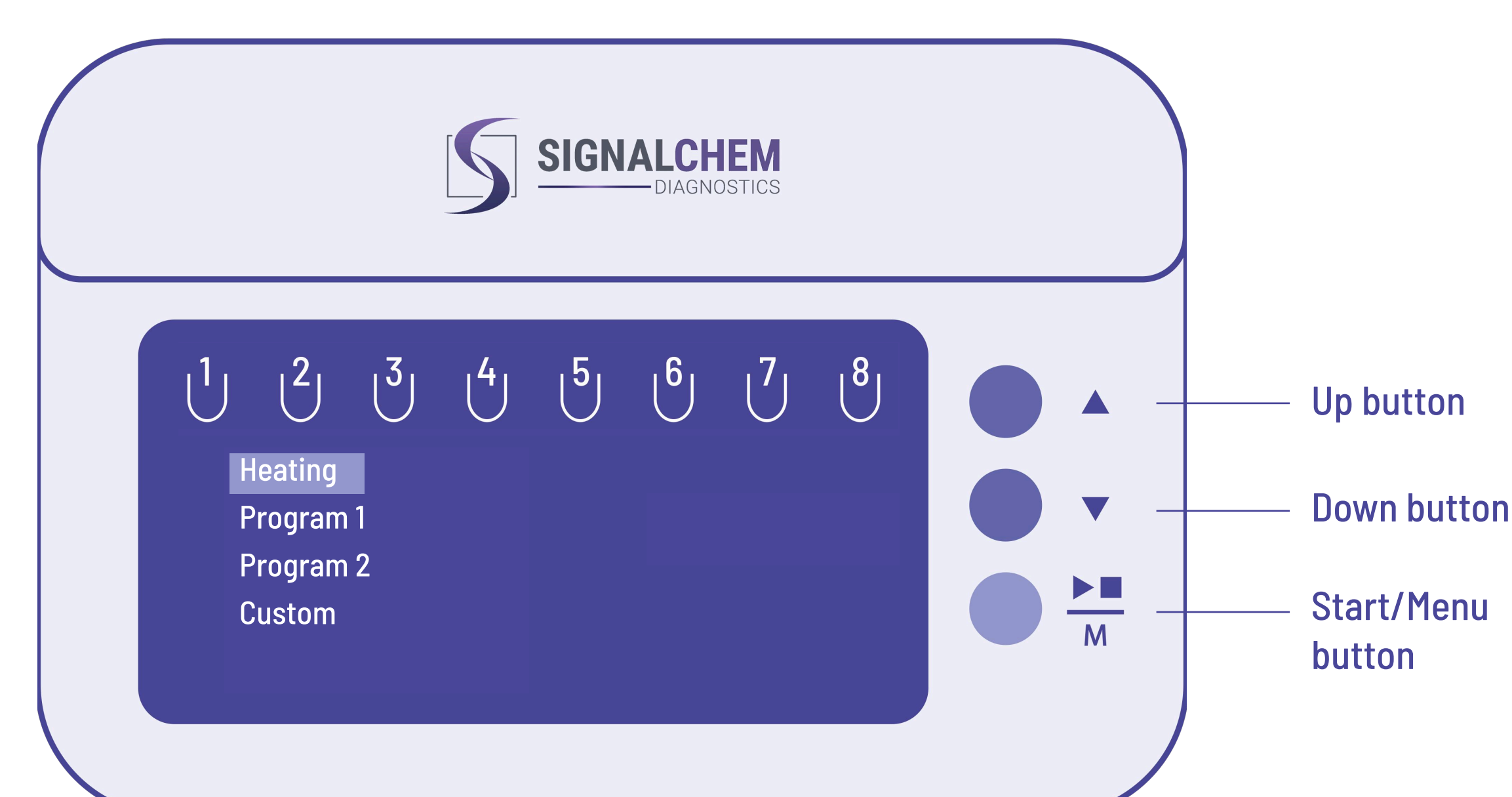
4. Add samples to master mix

- Set the adjustable pipette to 30 μ L and attach a new tip. Carefully open the lid for one sample. Transfer 30 μ L of the diluted sample (red-capped) into a master mix tube without touching the pellet. Close the master mix tube, discard the tip. Close the diluted sample tube. Repeat the steps above for all samples using new tips each time. Mix the master mix tube(s) contents by flicking to fully resuspend the pellet.
- CRITICAL: Check that all liquid is at the bottom of the tube and no large bubbles are present before proceeding to ensure accuracy.**
 - If a microcentrifuge is available, briefly centrifuge the tube to bring the entire volume to the bottom and eliminate any air bubbles.
 - Use a sharp downward wrist-flicking motion to bring the droplets to the bottom of the tube.
 - Lightly tap the tube(s) on a surface to bring the droplets to the bottom of the tube.



5. Run test in CRISPOT®

- Preheat the device by running the heating program on the device for 1 minute until the temperature reaches 60°C. Using the top and middle arrow buttons of the portable device, select "Heating". Run the heating program by pressing the play button. The program can be stopped by pressing the play/stop button twice.

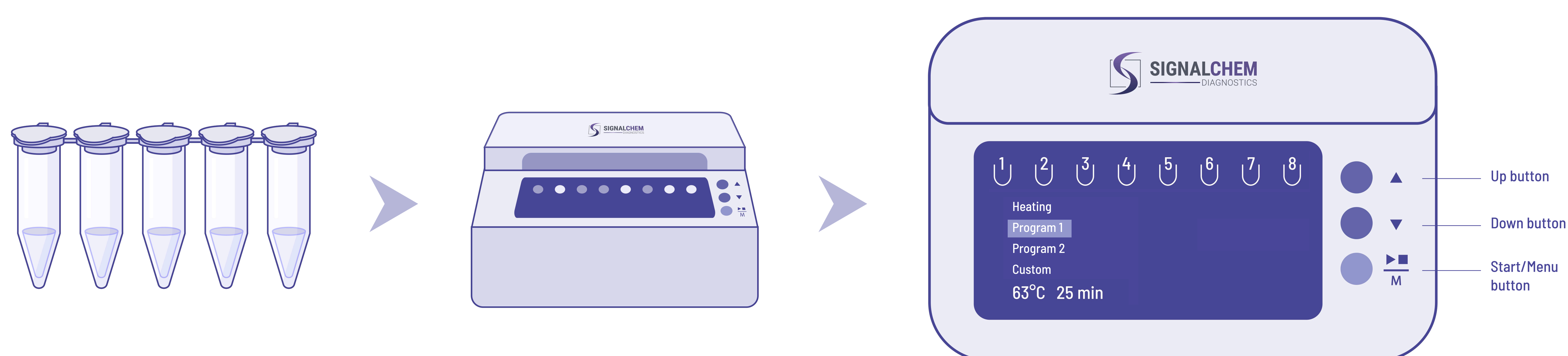


Sample Preparation: Full Comprehensive Visual Guide

Protocol (Continued)

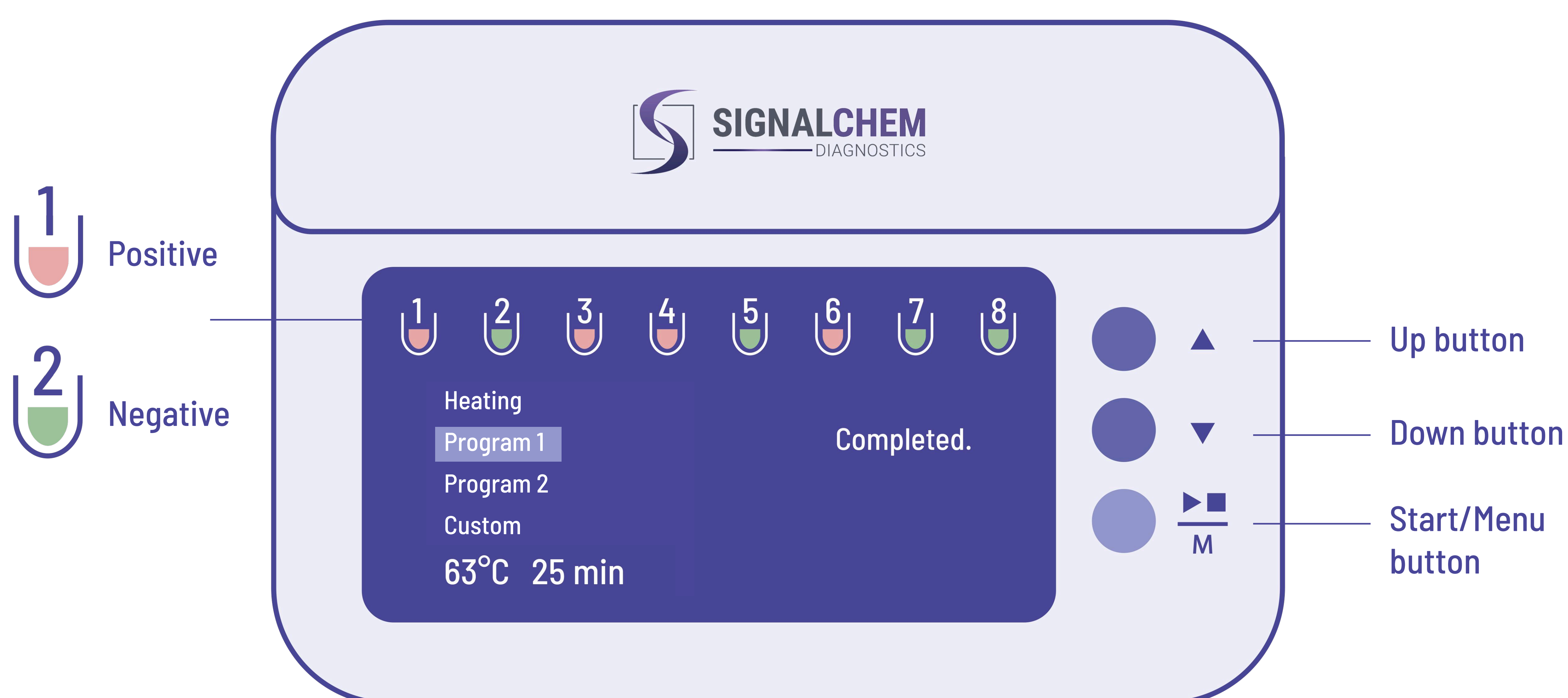
5. Run test in CRISPOT® (Continued)

- Place test strip (or portion if <8 samples) into CRISPOT® device. Using the top and middle arrow buttons of the portable device, select Program 1. Start the program by pressing the bottom “play” button.



6. Read results

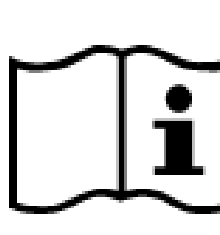
- After the run is completed, the infection status of each sample will be displayed: red for positive (detected) and green for negative (not detected). Record results immediately (write down or photograph), as they will be inaccessible if any buttons are pressed or the machine is turned off.



7. Dispose of materials

- DO NOT open master mix tubes once they have been tested in the CRISPOT®. All kit materials can be disposed of in regular garbage. Dispose of razors into sharps containers.

Index of Symbols

	Do not reuse		Manufacturer		Catalog number
	Temperature limit		Batch code		Consult instructions for use
	Caution		Use by		



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