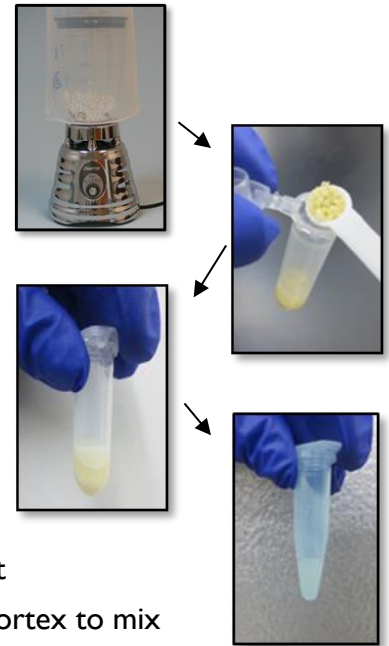


I. Prepare Sample using Sample Extraction Set 5

1. Pre-heat dry block to 95°C for at least 30 minutes
2. Grind a minimum of 200 soybeans (weight to be determined by average seed weight) in an Oster blender for 20 seconds; repeat 2 additional times for a total of three grinds
3. Shake MB9 bottle for 5 seconds to distribute particulate matter; add 600 µL of MB9 to a 2 mL clear tube supplied with Extraction Set 5
4. Using a clean grain scoop, add two unpacked level scoops of ground sample to clear tube and cap tightly; flick tube to ensure complete suspension of sample
5. Heat tube at 95°C for 6 minutes (±30 seconds)
6. **Remove tube and vortex for 5 seconds**
7. Centrifuge sample at 10,000 × g for 3 minutes (±30 seconds)
8. Add 100 µL of D4 buffer to a 1.5 mL blue tube supplied with Extraction Set
9. Add 100 µL of centrifuged sample to blue tube, avoiding particulates, and vortex to mix
10. 25 µL of this sample will be used in the assay



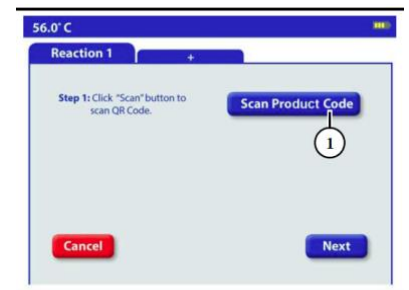
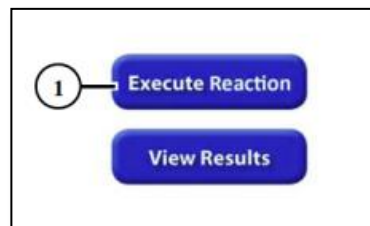
2. Prepare for Testing

1. **Turn on AmpliFire** Unit and allow to warm to 56°C
2. **Ensure** EnviroLogix GMO kit is at room temperature

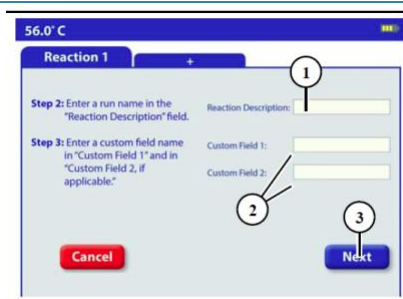


3. AmpliFire Setup

- After warmup, Select **“Execute Reaction”**
- Select **“Scan Product Code”** and scan barcode on Master Mix foil pouch using barcode camera on left side of reader
- Kit Identifier # will display
- Select **“Next”**

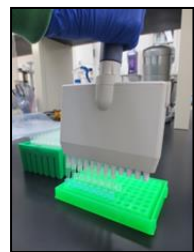
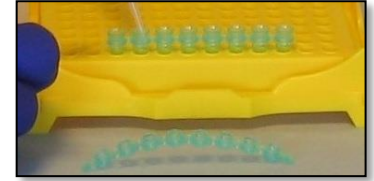


- Enter Run-Specific **“Reaction Description”** here
- Enter Custom Fields if applicable
- Select **Next**
- Enter Well-Specific info one well at a time or **“Finish”** to skip
- Select **Start** and AmpliFire will be ready to read strip tubes

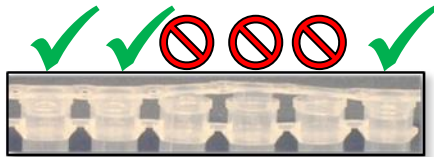
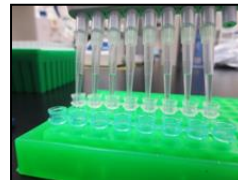


4. DNAbLe Assay Procedure

- Remove green Reaction Buffer strip tubes from the kit. Flick down prior to uncapping so that buffer is at bottom of tubes. Mark the left end for orientation.
- Transfer **25 μL of each sample** to the green Reaction Buffer vials. Use a fresh pipette tip for each transfer.
- **Recap tubes** and tap down or centrifuge to ensure all liquid is at the bottom of the tube



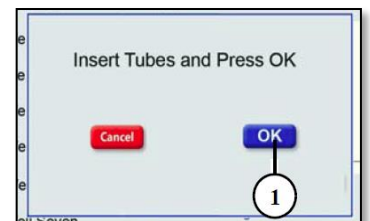
- Using multi-channel pipette, transfer **50 μL** to clear Master Mix tubes.
- Do **not** mix within the clear tube.



- Recap with **Flat Caps (seal completely!!)**.
- Mark left side of cap for orientation.
- Flick and tap down several times to remove air bubbles. **No bubbles** in bottom of tubes!



- Place capped and inspected clear strip tube in instrument and immediately press "OK"
- After 15 minutes, results will be displayed as **Not Detected (-)** or **Positive (+)**.
- After assay is complete, **remove run reaction strip tubes** discard in opened foil pouch. **Never open a completed reaction tube!**
- Export results from the Home Page (View Results/Export to USB)
- Clean up work area.



Materials Needed:

- Oster blender
- Precision pipettes to deliver 25-1000 μL (p-100 & p-1000)
- Pipette tips
- Dry heat block capable of $95 \pm 1^\circ\text{C}$ (with insert capable of holding 2 mL tubes)
- Vortex
- Microcentrifuge capable of $10,000 \times g$
- Timer