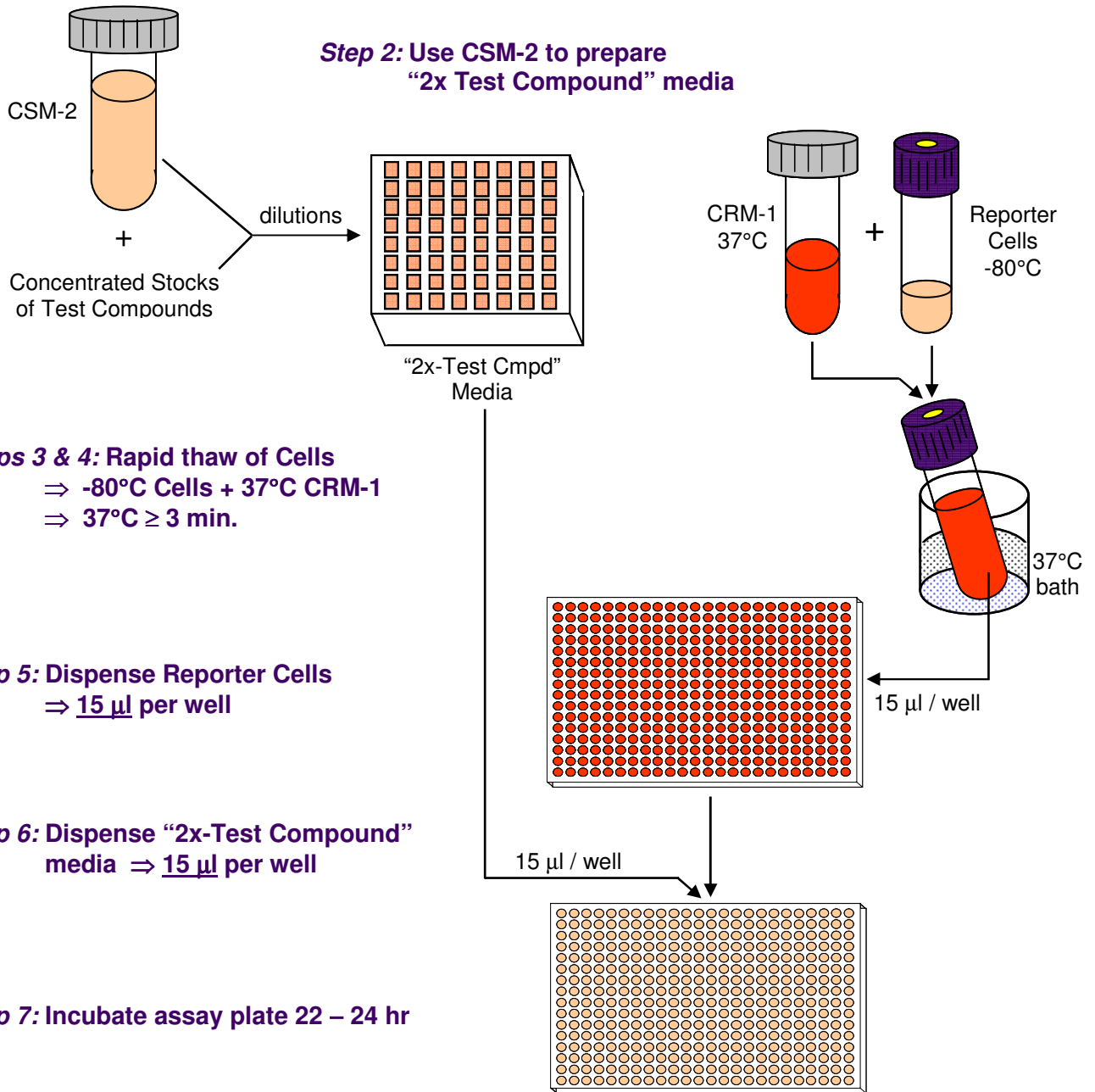


DAY 1

Step 1: Thaw CRM-1 ⇒ 37°C
Thaw CSM-2 ⇒ room temperature

Step 2: Use CSM-2 to prepare
“2x Test Compound” media



Steps 3 & 4: Rapid thaw of Cells
 ⇒ -80°C Cells + 37°C CRM-1
 ⇒ 37°C ≥ 3 min.

Step 5: Dispense Reporter Cells
 ⇒ 15 µl per well

Step 6: Dispense “2x-Test Compound” media
 ⇒ 15 µl per well

Step 7: Incubate assay plate 22 – 24 hr

Step 8: Transfer frozen “Detection Substrate” to 4°C to thaw overnight

Incubate
 37°C, 90% humidity, 5% CO₂

DAY 2

**Step 9: Equilibrate “Detection Substrate”
 to room temperature**

Step 10: Prepare Luminometer

If following Alternate Step 11A:

- Dispense Detection Substrate
 ⇒ 15 µl per well
- 30 minutes “reaction rest” at
 room temperature
- Read assay plate, ≤ 0.5 sec / well

If following Alternate Step 11B:

- Dispense Detection Substrate
 ⇒ 15 µl per well
- Read assay plate, ≤ 0.5 sec / well

