

# Ready Reference for Yeast and Mold PCR Assay

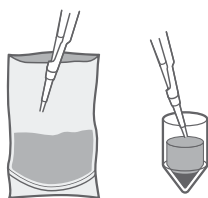
**1. Homogenize sample in 1:10 dilution according to the food type.**



**2. Determine sample volume to be tested.**

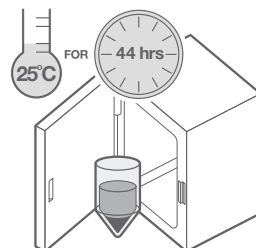
(See User Guide or table on back of this reference card.)

**3. Transfer sample to disrupter tube.**

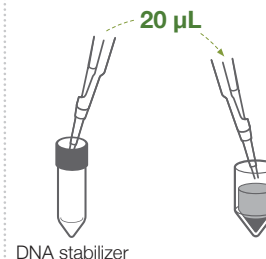


Pooled sample protocol requires triplicate disrupter tubes.

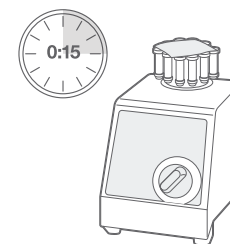
**4. Incubate disrupter tubes.**



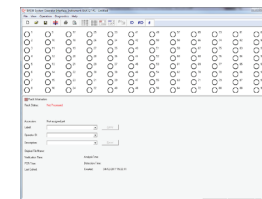
**5. Add DNA stabilizer to disrupter tubes.**



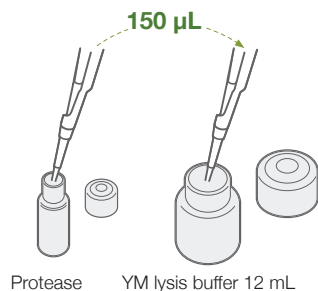
**6. Agitate in disrupter device.**



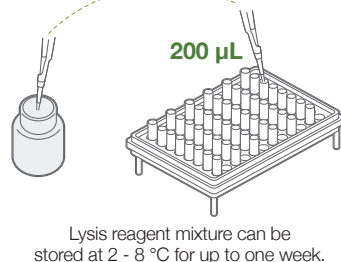
**7. Create a rack file.**



**8. Add protease to YM lysis buffer.**

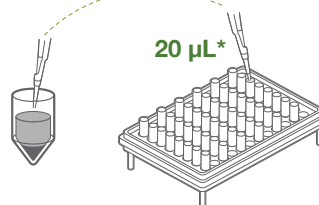


**9. Transfer lysis reagent made in step 8 to cluster tubes.**



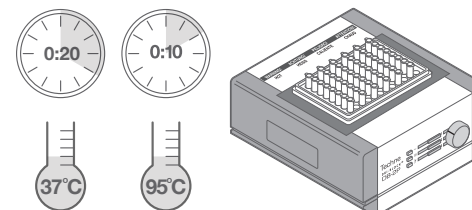
Lysis reagent mixture can be stored at 2 - 8 °C for up to one week.

**10. Transfer disrupted samples to cluster tubes.**



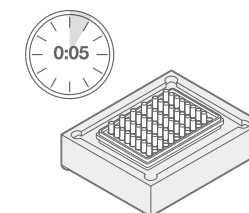
\*Pooled sample protocol requires pooled volumes from disrupter tubes into 1 cluster tube.

**11. Heat cluster tubes.**

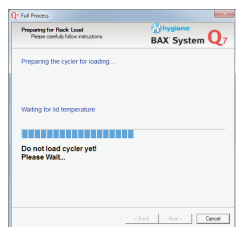


\* Steps 11 and 12 can also be performed using the Hygiena<sup>™</sup> Automated Thermal Block. See the Automated Thermal Block User Guide for details and instructions.

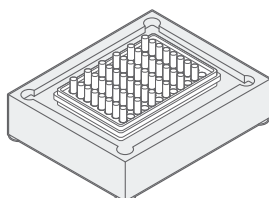
**12. Cool cluster tubes in cooling block.**



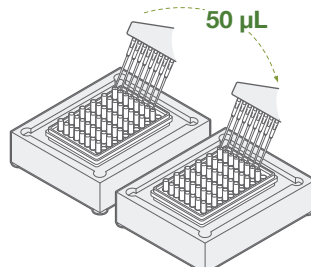
**13. Initialize cycler.**



**14. Arrange PCR tubes in cooling block.**



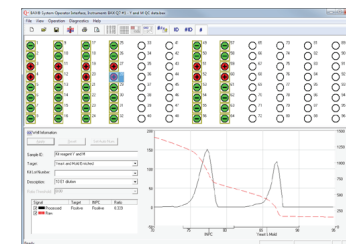
**15. Hydrate PCR tablets with 50 µl lysate from step 12.**



**16. Place tubes in cycler and run program.**



**17. Unload samples and review results on screen. See User Guide for details.**



# Ready Reference for Yeast and Mold PCR Assay

## Pooled Sample Protocol

This ultra-sensitive protocol uses pooled samples from three disrupter tube enrichment replicates for action levels of 10-50 cfu/g.

| If your action level is: | Then transfer this volume of homogenate to 3 disrupter tubes: | And pool these volumes of disrupted sample for testing: |
|--------------------------|---------------------------------------------------------------|---------------------------------------------------------|
| 10 cfu/g                 | 400 µL                                                        | 7 µL from 3 replicates                                  |
| 20 cfu/g                 | 200 µL                                                        | 7 µL from 3 replicates                                  |
| 50 cfu/g                 | 80 µL                                                         | 7 µL from 3 replicates                                  |

## Non-Pooled Sample Protocol

This protocol for yeast and mold testing can be used without pooling for action levels of 25 cfu/g or above.

| If your action level is: | Then use this volume of homogenate: |
|--------------------------|-------------------------------------|
| 25 cfu/g                 | 400 µL                              |
| 50 cfu/g                 | 200 µL                              |
| 100 cfu/g                | 100 µL                              |
| 500 cfu/g                | 20 µL                               |
| 1000 cfu/g               | 10 µL                               |