

# Ready Reference for Yeast and Mold PCR Assay

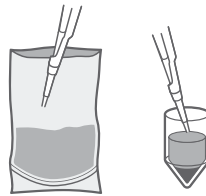
**1. Homogenize sample in 1:10 dilution according to the food type.**



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

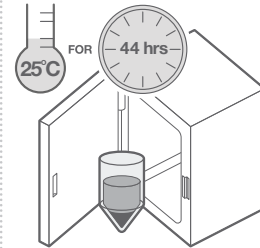
(See User Guide or table on back of this reference card.)

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

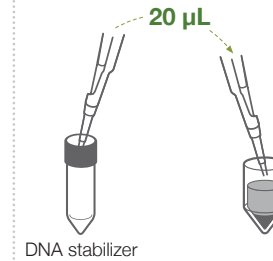


Pooled sample protocol requires triplicate disrupter tubes.

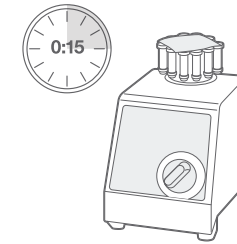
**4. Incubate disrupter tubes.**



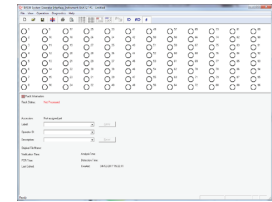
**5. Add DNA stabilizer to disrupter tubes.**



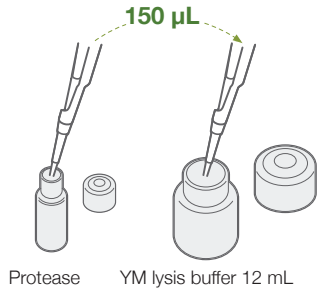
**6. Agitate in disrupter device.**



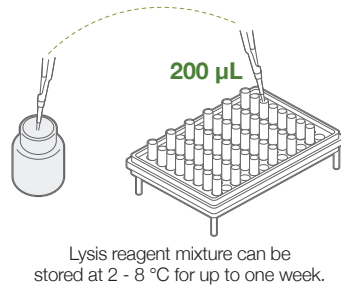
**7. Create a rack file.**



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

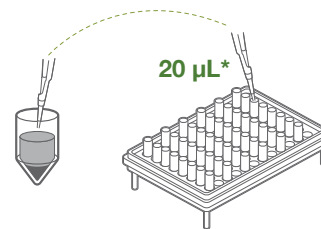


**9. Transfer lysis reagent made in step 8 to cluster tubes.**



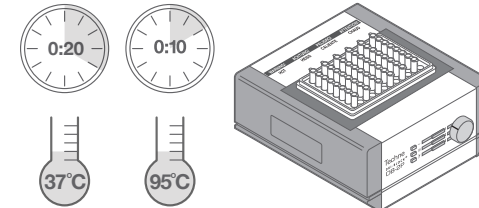
Lysis reagent mixture can be stored at 2 - 8 °C for up to one week.

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



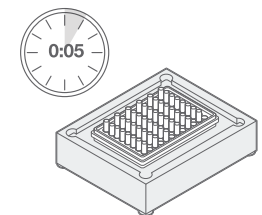
\*Pooled sample protocol requires pooled volumes from disrupter tubes into 1 cluster tube.

**11. Heat cluster tubes.**

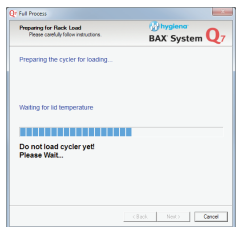


\* Steps 11 and 12 can also be performed using the Hygiena<sup>™</sup> Automated Thermal Block. See the Automated Thermal Block User Guide for details and instructions.

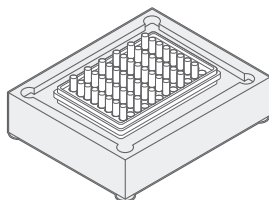
**12. Cool cluster tubes in cooling block.**



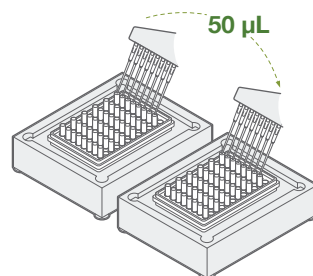
**13. Initialize cycler.**



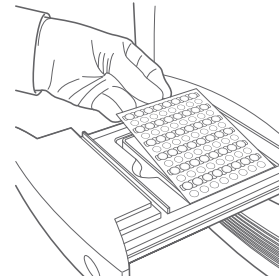
**14. Arrange PCR tubes in cooling block.**



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

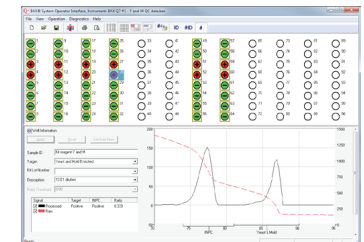


**16. Place tubes in cycler and run program.**



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

- Negative
- Positive
- Indeterminate
- Signal error



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## 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