# BAX Q7
# PCR System

# BAX® System Yeast & Mould assay Presentation

Agro-Food Diagnostics

AUSTRIA BULGARIA CROATIA CZECH REPUBLIC HUNGARY POLAND ROMANIA SERBIA SLOVAKIA SLOVENIA

# BAX® System for Detecting Yeast & Mold

- BAX® PCR assay detects yeast and mold
- Results in 48 hours vs. 5-7 days with culture methods
- Detects 10 cfu/g or greater after 44-hour enrichment
- **Direct testing without enrichment detects >500 cfu/g: Time to result. <5 hours**
- AOAC-RI approved on corn starch, yoghurt and milk based infant formula

**Yeast and Mold PCR assay**
96 tests per kit
Supplement kit – 96 disrupter tubes + 2 DNA stabilizer tubes

Cell disrupter device

# BAX® System Yeast & Mould threshold level protocols

- Direct protocol
- Enriched protocol

# Direct protocol

**Table 1. Sample volume calculations for direct testing**

| If Your Action Level Is: | And Your Desired Dilution Level Is: | Transfer This Much Homogenate: | To This Many Disruptor Tubes: |
|---|---|---|---|
| 250 cfu/g | 1:10 | 400 uL | 1 |
| 500 cfu/g | 1:10 | 200 uL | 1 |
| 1000 cfu/g | 1:10 | 100 uL | 1 |
| 2500 cfu/g | 1:10 | 40 uL | 1 |
| 5000 cfu/g | 1:10 | 20 uL | 1 |
| 10000 cfu/g | 1:10 | 10 uL | 1 |

# Enriched protocol

| Table 2. Sample volume calculations with provided media | | | |
|---|---|---|---|
| If Your Action Level Is: | And Your Dilution Level Is: | Transfer This Much Homogenate: | To This Many Disruptor Tubes: |
| 13 cfu/g | 1:10 | 400 uL | 2 |
| 25 cfu/g* | 1:10 | 400 uL | 1 |
| 50 cfu/g* | 1:10 | 200 uL | 1 |
| 100 cfu/g* | 1:10 | 100 uL | 1 |
| 500 cfu/g* | 1:10 | 20 uL | 1 |
| 1000 cfu/g* | 1:10 | 10 uL | 1 |
| | | | |
| 250 cfu/g | 1:100 | 400 uL | 1 |
| 500 cfu/g | 1:100 | 200 uL | 1 |
| 1000 cfu/g | 1:100 | 100 uL | 1 |
| > 5000 cfu/g | 1:100 | 20 uL | 1 |
| > 10000 cfu/g | 1:100 | 10 uL | 1 |

# BAX® System Yeast & mould threshold level

In order to find the right threshold level, use historical data and in-house BAX® System Yeast & Mould data

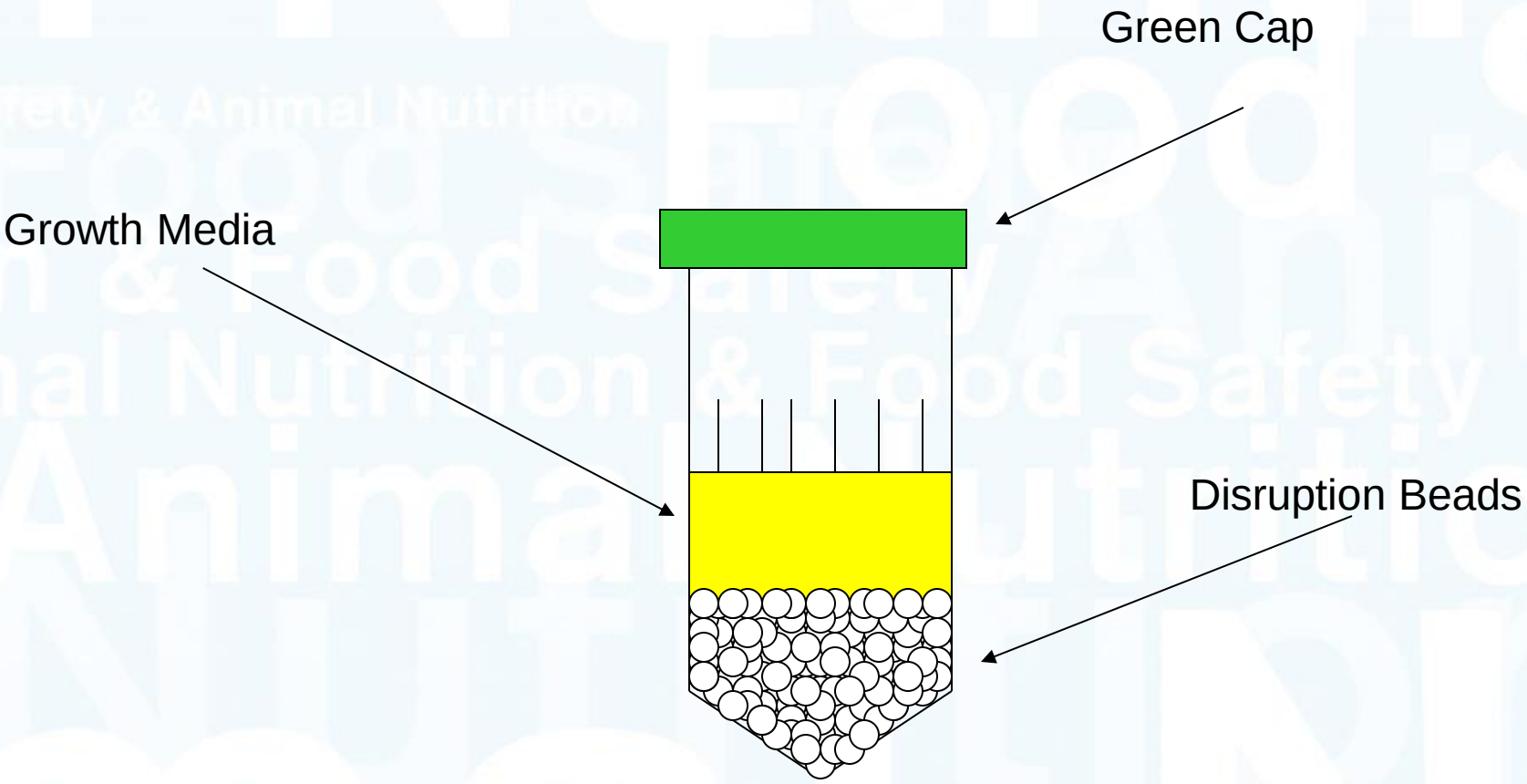
A threshold of e.g. 50 cfu/g will make you able to detect potential Y&M contamination FAST & at a very low level, reducing the level of re-calls:

Samples containing less than 50 cfu/g will be negative 

Samples at or above 50 cfu/g will be positive 

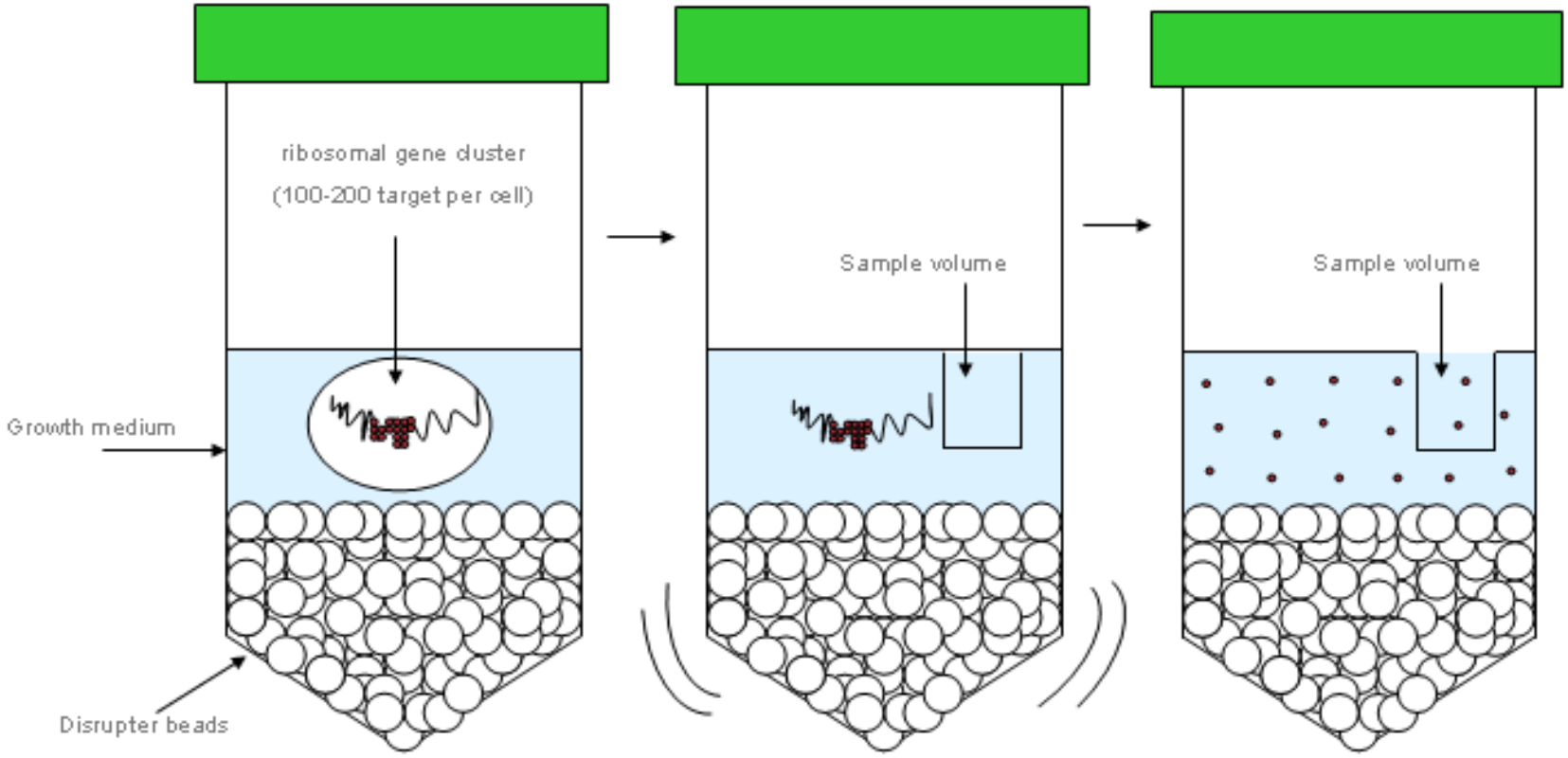
For the Direct protocol, the BAX® softwere will display a 

# BAX® yeast and mold disruption tube.



# Cell Disruption Fragments the DNA

Agitate the disrupter tube to fragment the gene cluster

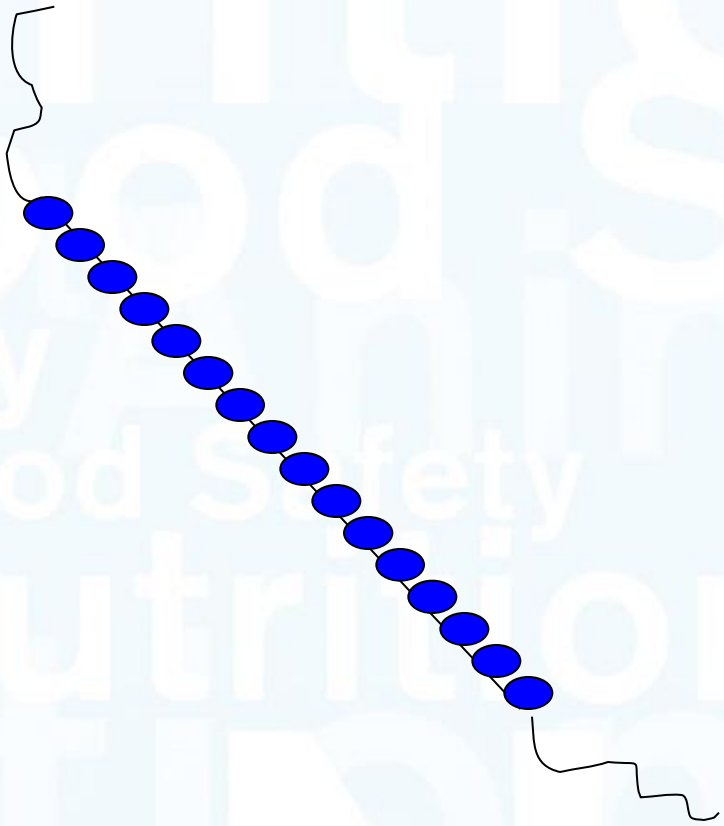


# BAX® system uses pan-fungal primers

Targets a portion of the ribosomal RNA (rRNA) gene using pan-fungal primers

The ribosomal gene cluster is repeated in tandem 100-200 times per fungal genome.

100-200 targets per cell, but closely linked on the same strand of DNA.



# BAX® System Ready Reference for Yeast & Mold PCR Assay

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

2. Determine sample volume to be tested.

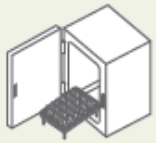
(See User Guide or table on back of this reference card.)

3. Transfer sample to disrupter tube.

Pooled sample protocol requires triplicate disrupter tubes.

4. Incubate disrupter tubes.

25°C for 44 hours

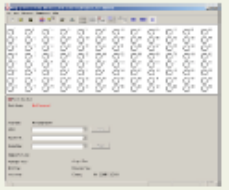


5. Add DNA stabilizer to disrupter tubes.

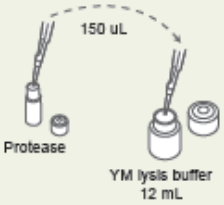

DNA stabilizer

6. Agitate in disrupter device.

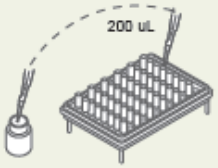

7. Create a rack file.



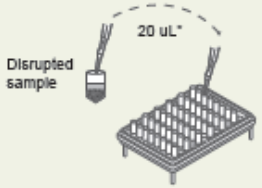
8. Add protease to YM lysis buffer.




9. Transfer lysis re-agent to lysis tubes.



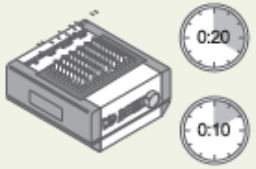

10. Transfer disrupted samples to lysis tubes.



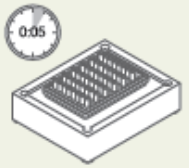

*Pooled sample protocol requires pooled volumes from disrupter tubes into 1 lysis tube.

11. Heat lysis tubes.

37°C for 20 minutes; 95°C for 10 minutes

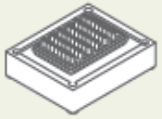



12. Cool lysis tubes in cooling block.

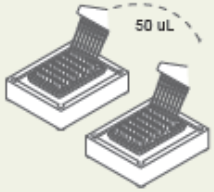



13. Warm up the cycler.

14. Arrange PCR tubes in cooling block.



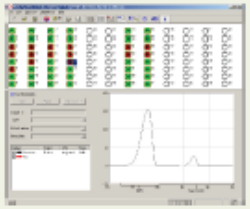
15. Transfer lysate to PCR tubes.




16. Place tubes in cycler and run program.

17. Review results on screen.

- Negative
- Positive
- Indeterminate
- Signal error



# BAX® Yeast and Mold assay

Tube contains a growth medium and disruption beads

↓

Sample homogenate is inoculated into disrupter tube.

- For direct test no incubation.
- For enriched test incubate @ 25C for 44 hours.

↓

20ul DNA Stabilizer is added

↓

Tubes bead beaten in Disruptor Genie for 15 min.

↓

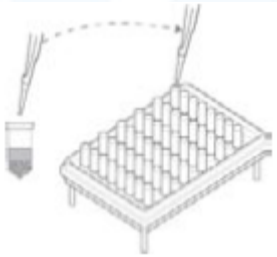
20ul of disrupted sample added to standard Bax™ lysis and processed as for gram negative bacteria.

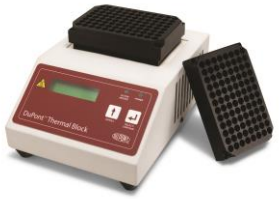
# Example: BAX® Y&M 50 cfu/g threshold protocol

Sample is diluted 1:10

Homogenize sample & transfer 200 uL to disrupter tubes & incubated 44 hours @ 25C

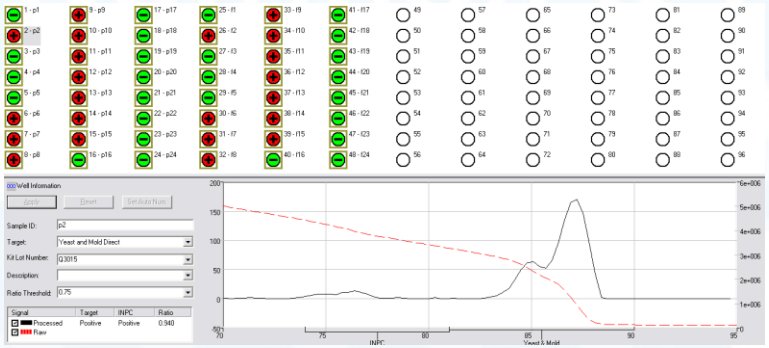
Add DNA stabilizer to disrupter tube and disrupt for 15 minutes in disrupter device





After disruption, 20 uL is added to cluster tubes and run through the lysis protocol in the automated DuPont Thermal Block

Hydrate the PCR tablets with 50 µL lysate, place the rack into the BAX® System instrument and run the program



Review results

# BAX® Yeast & Mould enriched samples

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 1 - almonds | 9 - hazelnuts | 17 | 25 | 33 | 41 | 49 | 57 | 65 | 73 |
| 2 - almonds | 10 - hazelnuts | 18 | 26 | 34 | 42 | 50 | 58 | 66 | 74 |
| 3 - almonds | 11 - chives | 19 | 27 | 35 | 43 | 51 | 59 | 67 | 75 |
| 4 - almonds | 12 - chives | 20 | 28 | 36 | 44 | 52 | 60 | 68 | 76 |
| 5 - almonds | 13 - chives | 21 | 29 | 37 | 45 | 53 | 61 | 69 | 77 |
| 6 - hazelnuts | 14 - chives | 22 | 30 | 38 | 46 | 54 | 62 | 70 | 78 |
| 7 - hazelnuts | 15 - chives | 23 | 31 | 39 | 47 | 55 | 63 | 71 | 79 |
| 8 - hazelnuts | 16 - maximum ... | 24 | 32 | 40 | 48 | 56 | 64 | 72 | 80 |

Well Information

Apply | Reset | Set Auto Num.

Sample ID: almonds

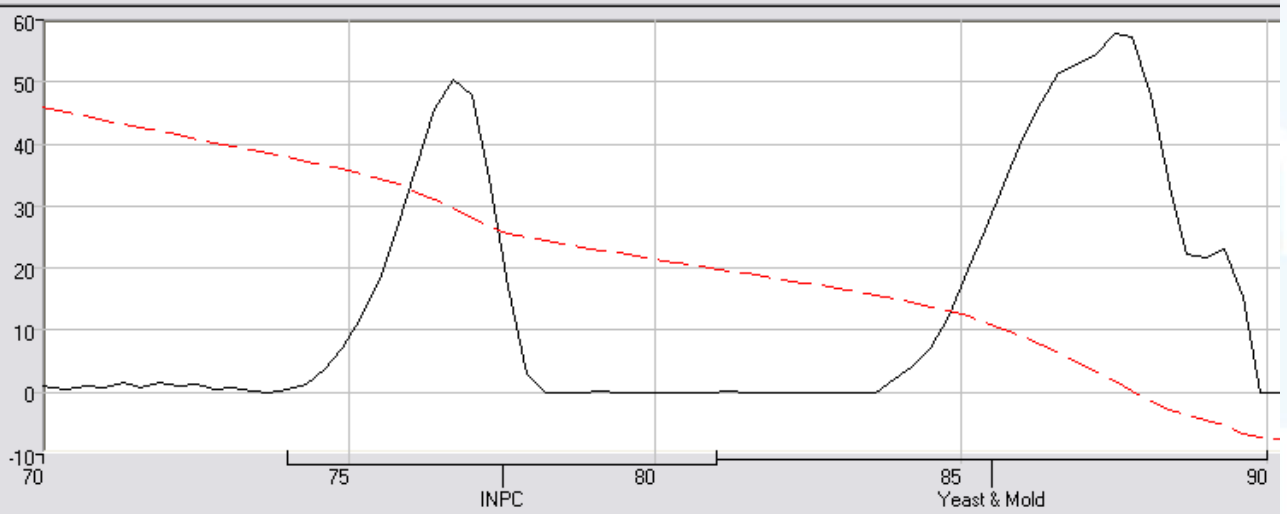
Target: Yeast and Mold Enriched

Kit Lot Number: Q8143

Description: almonds spiked with 100 cfu, 100 ul

Ratio Threshold: 0.00

| Signal | Target | INPC | Ratio |
|---|---|---|---|
| Processed | Positive | Positive | 0.713 |
| Raw | | | |




# Thanks for your attention!

**Thomas Pundy**
**NOACK & Co GmbH**