



Yeast and Mold Testing Solutions for Yogurt

Using the Soleris® Automated Detection System

Yogurt appears to be a simple product to test for yeast and mold, however a detailed examination of different brands and types reveals that yogurt is quite complex and that while automated analysis is possible, there is no “one size fits all” solution. In order to define an appropriate testing solution, the yogurt must be categorized by its characteristics and an appropriate testing procedure must be selected.

Yogurt Types	All Types of Yogurt	All Types except those with high probiotic loads	All Types except those with preservatives or high probiotic loads
Suggested Soleris Protocol	Pre-Enrichment	1:10 Dilution	1 gram Direct

Soleris Testing Protocols

The Soleris system offers three testing protocols for yogurt analysis which encompass a broad range in sensitivity requirements and yogurt types. The DYM-109C test vial is AOAC-RI approved. These three protocols are illustrated below (Table 1).

Table 1	Soleris Yogurt Testing Protocols		
	Pre-Enrichment ¹	1:10 Dilution ²	1 gram Direct ³
Sample Size	10 g into 90 mL	1 g into 9 mL OR 11 g into 99 mL	1 g
Pre-Enrichment Supplement	YI-110C (Optional, however it is required for probiotic yogurts)	NA	NA
Soleris Test Vial	DYM-109C	DYM-109C	DYM-109C
Soleris Test Vial Supplement	YI-110C	YI-110C	YMY-110Y
Pre-Enrichment Bottle	YM-EBY	N/A	N/A
Sensitivity ⁴	Presence/Absence, 10 grams in 72 hr	< 10 cfu/g in 48-72 hr	Presence/Absence, 1 gram in 72 hr ⁴

- 1. Pre-Enrichment** - All yogurt types can be tested using the Pre-enrichment protocol.
- 2. 1:10 Dilution** - All yogurt types except those with a high probiotic load can be tested using the 1:10 Specification Monitoring protocol.
- 3. 1 gram Direct** - Only yogurts without preservatives or without high probiotic loads are recommended. Product feasibility testing on actual customer production samples is highly recommended, as the Soleris testing parameters may need to be adjusted (example: probiotic concentration and type may require extended shut-eye to avoid matrix shut-eye DT) or pH adjustments may be necessary.
- 4. Sensitivity** - For the 1 gram direct protocol, some organisms may only be detectable at higher levels. This is due to the nature of yogurt, and its potential inhibitory effects on the growth of yeasts and molds.





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Yogurt Type Classification

Because there are many different types of yogurts with different characteristics that will impact yeast and mold testing, the following yogurt classification table provides guidance on the Soleris solution that should provide the most optimal test conditions (Table 2).

Table 2		Representative Brand Examples ¹	Protocol ²		
			Pre-enrichment	1:10 Dilution	1 gram Direct - Preservative free yogurts only
Yogurt Types	Contains Preservatives ³	Any brand/type/flavor may contain Potassium Sorbate, Sodium Citrate, Citric Acid (or other proprietary preservatives) in the yogurt, flavoring or fruit.	✓	✓	
	High Probiotic Load ⁴	Stonyfield Greek, Activia, Activia Greek	✓		
	High Probiotic Drinks ⁵	DanActive, Actimel, Danimals	✓	✓	✓
	Traditional (stirred) Yogurt	Yoplait original, Dannon regular, Dannon Fruit on the bottom, Dannon Light and Fit, Stonyfield YoBaby	✓	✓	✓
	Greek Yogurt	Chobani, Chobani Kids, Chobani Blended, Fage, Dannon Oikos, Yoplait Greek, Whipped, Yoplait Greek Plenti	✓	✓	✓
	Icelandic	siggis	✓	✓	✓
	Mediterranean	Liberte	✓	✓	✓
	Heat-treated post-fermentation (typically contain preservatives) ⁶	Yoplait Kids, Trix, Gogurt	✓	✓	
	Non-dairy Yogurt ⁷	Silk, Almond Dream, Stonyfield O'Soy	✓	✓	✓
	100% Preservative Free	Fage, Chobani, Stonyfield	✓	✓	✓



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- 1. Representative Brand Examples** - Brand examples are provided to show the varieties of yogurt, please check for preservatives first to indicate whether or not the 1 gram direct protocol is applicable. Note that Representative Brand Examples are provided for reference only. All product and company names are trademarks™ or registered® trademarks of their respective holders. Use of them does not imply any affiliation with or endorsement by them or Neogen Corporation.
- 2. Protocols** - Varieties in flavor, whipped, low calorie, sugar substitution, etc. may have an impact on the need for pH adjustment if the 1 gram direct protocol is selected. The viscosity of a yogurt should not be of significant impact to selecting a protocol. For drinkable or smoothie type yogurts, a protocol should be selected according to preservative or probiotic characteristics in Table 2.
- 3. Contains preservatives** - Preservative levels are not typically available, as this is proprietary information. Some product lines may contain preservatives in a specific flavor, and not in others. Yeasts and molds are not typically a concern for these yogurts. Any preservatives in yogurt may cause a delay in detection times. The 1 gram direct protocol is not recommended for these yogurts, due to the selectivity of the YMY-110Y supplement. There may be an increase in toxicity from the combined effects of preservatives and the YMY-110Y supplement, thus suppressing Y&M growth.
- 4. High probiotic load** - Probiotic yogurts typically state “Contains live and active cultures” and list the names of a few strains. “High probiotic load” is a relative term, and yogurt producers should know their probiotic level, relative to other yogurts. All dairy based yogurts are made with live cultures, but some may be heat treated or the cultures are not hardy enough to survive in high levels by the time the product reaches the consumer. To mitigate these factors, the Pre-Enrichment protocol is recommended for yogurts rich in probiotics because it specifically promotes selective growth of the yeast and mold colonies.
- 5. High Probiotic Drinks** - The 1 gram direct protocol may work for some high probiotic drinks, but not all. It truly depends on the activity of the lactics in the drink. These type of products will have to be tested for feasibility on a case-by-case basis by Neogen Technical Services.
- 6. Heat treated post-fermentation yogurts (typically contain preservatives)** - Pasteurized yogurt-like products are manufactured using milk that was inoculated with bacterial cultures, and left to ferment until the desired consistency and flavor is achieved, then pasteurized. Therefore, there are no live cultures present in these yogurt products. However, these yogurts typically contain preservatives, and therefore the 1 gram direct protocol is not recommended.
- 7. Non-dairy yogurt** - Non-dairy yogurts do not contain live and active cultures, however there may be entrained CO₂ so a parameter adjustment may be necessary.



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Testing Procedures using Pre-enrichment

1. Determine if your yogurt YM-EBY pre-enrichment bottle will require supplementation. Supplement is recommended for high probiotic yogurts.
2. If necessary, add 2.5 mL rehydrated YI-110C to each YM-EBY bottle. Mix well.
3. Weigh 10g yogurt into 90 mL YM-EBY bottle. Shake vigorously to distribute yogurt.
4. Open cap one quarter turn, and incubate YM-EBY pre-enrichment bottle for 48 hours at 25°C.
5. After pre-incubation, tighten cap and shake vigorously for 10 seconds to ensure distribution of organism growth.
6. Allow DYM-109C vials to equilibrate to room temperature before use.
7. Add 0.6 mL rehydrated YI-110C supplement to each vial. Mix well.
8. Pipette 1 mL of pre-incubated YM-EBY bottle to each test vial. Mix well.
9. Open cap one quarter turn, and put into instrument set at 25°C.
10. Run test for 24 hours.

Testing Procedures using 1:10 Dilution

1. Allow DYM-109C vials to equilibrate to room temperature before use.
2. Add 0.6 mL rehydrated YI-110C supplement to each vial. Mix well.
3. Create a 1:10 dilution for yogurt by adding 1 g to 9 mL Butterfields Phosphate Buffer (or 11 g into 99 mL).
4. Mix well.
5. Pipette 1 mL of 1:10 dilution into DYM-109C vial. Mix well.
6. Open cap one quarter turn, and put into instrument. Set at 25°C.
7. Run test for 48-72 hours.

Testing Procedures using 1 gram Direct

1. Prior to testing, determine if test vials require a pH adjustment.
 - a. Add 1 g of yogurt to DYM-109C vial. Shake vigorously for 10 seconds to distribute yogurt.
 - b. Record pH. Desired vial pH range is 5.6 to 5.8.
 - c. If necessary, slowly titrate 1N NaOH until pH falls between 5.6 and 5.8.
2. Allow DYM-109C vials to equilibrate to room temperature before use.
3. Add pH adjustment (if necessary). Mix well.
4. Add 0.3 mL rehydrated YMY-110Y supplement to each vial. Mix well.
5. Weigh 1 g yogurt into each vial. Immediately shake vigorously for 10 seconds to distribute yogurt.
6. Open cap one quarter turn, and put into instrument set at 25°C.
7. Run test for 72 hours. Note: Test duration may need to be extended due to the nature of typical contaminant present. Consult with Neogen Technical Services for further information.



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