isolationbio

Microbial Isolation Service

Sample requirements and technical information

Table of Contents

Introduction

This document outlines the technical requirements and information for Isolation Bio's microbial isolation and cultivation service. Isolation Bio handles all aspects of the isolation and cultivation process, returning research ready microbial isolates to you in 96-well plate glycerol stocks. Microbial isolation is performed on the Prospector® high-throughput microbial isolation and cultivation platform.

Full project scope and expected results will be discussed during the scientific consultation call prior to shipment of samples. To expedite your service, we recommend familiarizing yourself with the information in this document ahead of the consultation call.

Validated Prospector® Media Types

The following aerobic and anaerobic media types have been fully validated for use on the Prospector. Additional media types not listed here may still be suitable. Please reach out to us via the contact form to discuss your specific project and preferred media.

Validated Prospector Media | **AEROBIC**			
Name	**pH**	**Full name**	**Source**
TWYE	~7	Tap water yeast extract	Prepared internally, DSMZ 1625
LB	7.2 ± 0.2	Luria-Bertani broth	Teknova L8050
BHI (50/100%)	7.4 ± 0.2	Brain Heart Infusion broth	Teknova B9993
R2A	7 ± 0.2	Reasoner's 2A	Teknova R0005
TYG	7.4 ± 0.2	Tryptone Yeast Extract Glucose	Prepared internally, ATCC 741
TSB	7.3 ± 0.2	Tryptic Soy Broth	BD 211825
YMB	6.8 ± 0.2	Yeast Mannitol Broth	Millipore Y3377

Validated Prospector Media | **ANAEROBIC**			
Name	**pH**	**Full name**	**Source**
GAM	7.3 ± 0.1	Gifu Anaerobic broth	HiMedia M1801
mGAM	7.3 ± 0.2	Modified Gifu Anaerobic broth	Himedia M2079
BHI	7.3 ± 0.3	Brain Heart Infusion (includes Heme/Vit K)	Anaerobe Systems AS-875
BHI	7.4 ± 0.2	Brain Heart Infusion	Millipore 53286
BRU	7.1 ± 0.2	Brucella Broth	Anaerobe Systems AS-105
YCFAC	6.8 ± 0.3	Yeast Casitone Fatty Acids broth with carbohydrates	Anaerobe Systems AS-680
MTGE	7.1 ± 0.4	Anaerobic Enrichment broth	Anaerobe Systems AS-780
PYGB	7.2 ± 0.3	Peptone Yeast Extract broth with Glucose	Anaerobe Systems AS-822
FAB	7.2 ± 0.2	Fastidious Anaerobe broth	Neogen NCM0199A
RCB	6.8 ± 0.3	Reinforced Clostridial broth	Anaerobe Systems AS-606
B&B	5.9 ± 0.2	Bryant and Burkey Media	HiMedia GM1385
Mega	7.1 ± 0.2	Mega media	Prepared internally
M2GSC	6.3	M2GSC media with 30% clarified rumen fluid	Prepared internally, ATCC2857

Sample Requirements

Sample and matrix description

Describe sample source and state. The following information should be included:

- Is the sample fresh or frozen?
- Is it in original form or diluted in a buffer?
- What buffer/media is it stored in (if any)
- Percent dilution from original form
- Does it contain glycerol

Here are example descriptions of typical sample submissions:

- 500 µL of human fecal, undiluted, flash frozen and stored at -80°C after collection. No buffer or glycerol.
- 300 g of mouse fecal pellets homogenized in anaerobic PBS buffer with 25% glycerol and frozen at -80°C. Sample diluted 1:10 from original pellets.
- 5 g of garden soil stored at 4°C for 3 days before shipping. No buffer or glycerol.
- 1 mL of rumen fluid enrichment with 25% glycerol for final dilution of 1:5, stored at -80°C.

Concentration (cells/mL)

Ideal sample concentration is at least 1e7 cells/mL. Lower concentrations may be accommodated with further discussion.

Amount

Recommended amounts for typical sample types:

- 1 mL of liquid enrichments/cultures, diluted fecal, rumen, or other “slurry” type samples
- 0.2 to 1 g of solid fecal samples
- 2 to 5 g soil

Media

Indicate preferred media choices. See list for Prospector-validated media (Page 2).

Target number

Indicate the targeted number of isolates. Isolation Bio cannot guarantee the number of isolates generated but will aim for a particular quantity.

Packaging and Shipping Requirements

Sample names

Clearly label sample containers (Eppendorf tube, cyro vial, etc.) with sample name/ID. Please include a list of the sample names/IDs on a separate sheet of paper.

Shipping method

Ship no later than Wednesday with overnight FedEx and provide Isolation Bio with tracking number. If frozen include plenty of dry ice, if fresh/unfrozen include cold packs as needed. Protect sample from breakage and spillage during shipping.

Shipping address

Isolation Bio Services Department
733 Industrial Rd
San Carlos, CA, 94070

Anaerobic samples

For anaerobic samples the customer is responsible for oxygen exposure of sample up until delivery and Isolation Bio acknowledgement of receiving sample.

Isolates Returned from the Prospector

Glycerol stocks

Isolates are prepared as 25% glycerol stocks in 200 µL volumes. Upon request Isolation Bio can prepare duplicate glycerol plates which will be 100 µL volume in each. Empty wells for downstream controls can be left in the 96-well plates by request.

Plates and seals

Glycerol stocks are prepared in 96-well culture plates and sealed with thick -80˚C-safe plastic seals. Heavy foil seals can be used upon request.

Plates for gDNA

Upon request Isolation bio can prepare duplicates in PCR plates for gDNA extraction prior to the addition of glycerol. The plates will be covered with foil seals.

Turbidity

Isolates will have visible turbidity and/or a growth indicator dye color change when collected from the Prospector transfer plates, the OD600 values will not be quantified. Some isolates may be in floc form or as small colonies suspended in the liquid.

Viability

Isolate cultures generated from complex samples under identical conditions will have a wide range of cell densities and will be at different stages of growth (stationary, exponential, death phase). Isolation Bio cannot guarantee viability of all isolates.

Purity

The cell suspension will be loaded at a concentration onto the array such that 90 – 95% of the cells that seed a nanowell will be single cells. This is based on statistics and not biology. To confirm purity of isolates we recommend sequencing.

Frequently Asked Questions

Can you use my prepared media?

- Yes. Provide 500 mL (volume may differ based on project goals) in two or more bottles. Media must be sterile. Indicate pH and composition. If the media has not been used with the Prospector previously we will internally validate the media before using it in the Prospector workflow. Pack very well when shipping to prevent contamination, breakage and spillage. If anaerobic, indicate if resazurin and cysteine are included and if degassing is required.
- We also accept media in powdered form that we can prepare on site.

Can I provide additional compounds that can be added to a general media?

Yes, we can add antibiotics, sporulation factors, growth factors, vitamins, etc that you provide to our general media.

What media are incompatible?

To date, only LMS and LMRS are fully incompatible. Low and high pH are case-dependent and will require validation.

Can you prepare media for me from a recipe?

Yes, but case dependent.

What if my selective media of choice is incompatible?

We can load the Prospector arrays with a more general media (e.g., BHI) and then transfer from the arrays to microtiter plates that have the selective media (e.g., LMRS). The number of successful transfers will be low, but what does transfer will likely be your target phenotype.

My media is very dark, will it be compatible?

Array growth is monitored via fluorescent signals and the color of the media will not interfere. Very dark media, such as those with high percentages of rumen fluid, cannot be used in the downstream 96-well transfer plates since we rely on visible turbidity and/or colorimetric growth indicator dye to identify growth.

If not all the isolates grow when they are transferred from the array to the 96-well microtiter plate, will you consolidate the isolates that do grow before preparing glycerol stocks?

Yes, with the exception of only a few isolates not growing. These plates will be prepared as is, with the non-growth wells marked.

Can you enrich my sample before loading?

Yes. Provide enrichment details and any specialized reagents (e.g., sporulation factors, non-standard antibiotics, carbon sources, etc). Enrichment will skew the community towards phenotype being enriched.

I am having trouble reviving my freezer stocks, what can I do?

- Use a larger volume of inoculum of thawed and mixed freezer stock (e.g., 20 µL)
- Spread on rich media agar plates, e.g., YCFA- or BHI-blood agar
- Incubate for longer period of time (one week or longer)
- If you know the identity of the isolate, revive under media/incubation conditions optimal for that species