PRODUCT SPECIFICATION SHEET

Tryptone Peptone Glucose Yeast Extract Broth Base w/o Trypsin (DM1002)

Intended Use
Tryptone Peptone Glucose Yeast Extract Broth Base w/o Trypsin (DM1002) is recommended for testing toxicity of Clostridium botulinum cultures.

Product Summary and Explanation
Clostridium botulinum is a gram-positive, anaerobic, spore-forming, rod-shaped bacteria that produces several toxins. The best known are its neurotoxins, subdivided in types A-G, that cause the flaccid muscular paralysis seen in botulism. Botulism poisoning can occur due to improperly preserved or home-canned, low-acid food that was not processed using correct preservation times and/or pressure. *Clostridium botulinum* is a soil bacterium. The spores can survive in most environments and are very rigid to kill. They can survive the temperature of boiling water at sea level, thus many foods are canned with a pressurized boil that achieves an even higher temperature, sufficient to kill the spores. *C. botulinum* cultures fall into three distinct groups by properties other than the toxin type they produce, with each group composed only of strains having similar cultural and physiological characteristics. Proteolysis i.e. ability to digest coagulated egg white or meat, is one of the differentiating characteristic. Toxins of non-proteolytic types do not manifest maximum potential toxicity until they are activated. Therefore food supernatant, liquid food, TPGY Broth or cooked meat cultures are treated with trypsin for activation. Toxins of proteolytic types do not need such activation. Optimum temperature for growth and toxin production of proteolytic strains is close to 35°C; for nonproteolytic strains it is 26-28°C. Non-proteolytic types B, E, and F can produce toxin at refrigeration temperatures (3- 4°C). Toxins of the nonproteolytics do not manifest maximum potential toxicity until they are activated with trypsin; toxins of the proteolytics generally occur in fully (or close to fully) activated form.
Tryptone Peptone Glucose Yeast Extract (TPGY) Broth is formulated as per recommendation of APHA,[1] for the determination of toxicity of *Clostridium botulinum* cultures in food.

Principles of the Procedure
Tryptone Peptone Glucose Yeast Extract Broth Base w/o Trypsin contains Casein enzymic hydrolysate, peptic digest of animal tissue and yeast extract which provides the nitrogen and carbon source to the medium. Growth of the bacterium can be prevented by high acidity, high ratio of dissolved sugar, high levels of oxygen, and very low levels of moisture or storage at temperatures below 3°C (38°F) for type. Dextrose serves as fermentable carbohydrate. Sodium thioglycollate serves as a reducing agent.

Formula / Liter

Ingredients	Gms / Liter
Casein enzymic hydrolysate	50.00
Peptic digest of animal tissue	5.00
Yeast extract	20.00
Dextrose	4.00
Sodium thioglycollate	1.00
Final pH: 7.0 ± 0.2 at 25°C	
Formula may be adjusted and/or supplemented as required to meet performance specifications	

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Precautions

1. For Laboratory Use only.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.

Directions

1. Suspend 80 grams of the medium in one liter of distilled water.
2. Heat if necessary to dissolve the medium completely.
3. Autoclave at 121°C, 15 psi pressure, for 15 minutes / validated cycle.
4. Refrigerate the sterile medium until use.
5. Before inoculation add 1.5% filter sterilized trypsin solution to a final concentration of 0.1% if desired.

Quality Control Specifications

Dehydrated Appearance	Cream to yellow homogeneous free flowing powder
Prepared Medium	Yellow coloured clear solution without significant precipitate.
Reaction of 8.00% Solution	pH : 7.0 ± 0.2 at 25°C
Gel Strength	Not Applicable

Expected Cultural Response: Cultural characteristics observed under anaerobic condition, after an incubation at 26-28°C for upto 7 days.

Sr. No.	Organisms	Results to be achieved	
		Inoculum (CFU)	Growth
1.	*Clostridium botulinum ATCC 25763*	50 - 100	good-luxuriant

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

1. Presumptive *C. botulinum* cultures are inoculated into Tryptone Peptone Glucose Yeast Extract Broth Base w/o Trypsin, for the non-proteolytic types and Cooked Meat Medium (DM248) for the proteolytic types.
2. Incubate inoculated tubes for 7 days and then test for toxin.
3. If there is no growth after 7 days of incubation, incubate for an additional 10 days to permit possible delayed germination of spores of *C. botulinum* before discarding.
4. Toxins of non-proteolytic types do not manifest maximum potential toxicity until they are activated. Therefore food supernatant, liquid food, TPGY Broth or cooked meat cultures are treated with trypsin for activation. Toxins of proteolytic types do not need such activation.

Results

Refer to appropriate references and test procedures for interpretation of results.

Storage

Store the sealed bottle containing the dehydrated medium at 10 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light.

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Expiration

Refer to the expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if the appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitations of the Procedure

1. For identification, organisms must be in pure culture. Morphological, biochemical and/or serological tests should be performed for final identification.
2. Consult appropriate texts for detailed information and recommended procedures.

Packaging

Product Name : Tryptone Peptone Glucose Yeast Extract Broth Base w/o Trypsin
Product Code : DM1002
Available Pack sizes : 100gm/ 500gm

References

1. Vanderzant C. and Splittstoesser D. F., (Eds.), 1992, Compendium of Methods for the Microbiological Examination of Foods, 3rd Ed., APHA, Washington, D.C.

Further Information

For further information please contact your local MICROMASTER Representative.

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