

## Product Data Sheet

### **DIPHTHERIA VIRULENCE AGAR BASE**

**Product No.** GB-DCM-00177-1A

### **INTENDED USE**

For determination of toxigenicity of *Corynebacterium diphtheriae*.

### **PRODUCT SUMMARY**

*Corynebacterium diphtheriae* is a principle human pathogen and owes its pathogenicity to the production of a potent exotoxin active on a variety of tissue including heart muscles and peripheral nerves. Toxin diffusing from a streak culture of suspected *C. diphtheriae* is demonstrated by the formation of a white line of precipitate where it meets with diphtheria antitoxin diffusing from a strip of filter paper embedded in the agar. In vitro toxigenicity (virulence) of *C. diphtheriae* was first described by Elek. Eleks technique was further improved by King, Frobisher and Parsons by the use of a standardized medium. This medium gave results comparable with animal inoculation test. Also it was found that proteose peptone supported toxin production in addition to maintaining the consistency of results. Hermann et al developed a non-serum based enrichment to overcome the irregularities encountered during the usage of horse, sheep or rabbit serum based enrichments. These non-sera based enrichments consist of Acicase, tween 80 and glycerol. Upon incubation of the inoculated plate, a line of precipitin is observed for toxigenic strains.

### **Product Specifications**

<b>Ingredients</b>	<b>Gms / Ltr</b>
Proteose peptone	20.000
Sodium chloride	2.500
Agar	15.000

### **PRINCIPLE**

The medium consists of proteose peptone which provides the carbon and nitrogen sources required for good growth of a wide variety of organisms and also for toxin production. Sodium chloride maintains the osmotic balance of the medium. Agar is incorporated as the solidifying agent. Potassium tellurite inhibits most gram-negative bacteria except *Corynebacterium* species, *Streptococcus mitis*, *Streptococcus salivarius* and *Enterococci*. *Staphylococcus epidermidis* may exhibit growth. False positive results may also be encountered. Therefore, a positive control has to always be run in parallel. *Corynebacterium ulcerans* and *Corynebacterium pseudotuberculosis* may also produce line of precipitation.

Microorganism	ATCC	Inoculum (CFU/ml)	Growth	Recovery	Line of precipitin	Incubation Temperature	Incubation Period
Bacillus subtilis subsp. spizizenii	6633	$\geq 10^4$	Luxuriant	0%	-	35-37°C	18-40 Hours
Corynebacterium diphtheria	8028	50-100	Luxuriant	$\geq 70\%$	Positive	35-37°C	18-40 Hours
Corynebacterium diphtheriae	8032	50-100	Luxuriant	$\geq 70\%$	Positive	35-37°C	18-40 Hours
Staphylococcus aureus	25923	$\geq 10^4$	Luxuriant	0%	-	35-37°C	18-40 Hours
Staphylococcus epidermidis	12228	50-100	Luxuriant	0.10%	-	35-37°C	18-40 Hours

#### INSTRUCTION FOR USE

- Dissolve 37.5 grams in 1000 ml purified/distilled water.
- Heat to boiling to dissolve the medium completely.
- Sterilize by autoclaving at 15 psi pressure (121°C) for 15 minutes. Cool to 55-60°C.
- Aseptically add 2 ml sterile KL Virulence Enrichment and 0.5 ml sterile 1% Potassium Tellurite to a 100 mm Petri plate and quickly add 10 ml of sterile Diphtheria Virulence Agar Base. Before the medium solidifies, place a filter paper strip saturated with potent Diphtheria antitoxin across the diameter of the plate. Allow the strip to sink to the bottom of the plate. Inoculate the plate with heavy inoculum across the strip.



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### **QUALITY CONTROL SPECIFICATIONS**

Appearance of Powder: Cream to yellow homogeneous free flowing powder.  
Appearance of prepared medium: Medium amber coloured, slightly opalescent gel forms in Petri plates.  
pH (at 25°C) : 7.8±0.2

### **STORAGE**

Dehydrated powder, hygroscopic in nature, store in a dry place, in tightly-sealed containers between 25-30°C and protect from direct sunlight. Under optimal conditions, the medium has a shelf life of 4 years. When the container is opened for the first time, note the time and date on the label space provided on the container. After the desired amount of medium has been taken out replace the cap tightly to protect from hydration.

**Product Deterioration:** Do not use if they show evidence of microbial contamination, discoloration, drying or any other signs of deterioration.

### **DISPOSAL**

After use, prepared plates, specimen/sample containers and other contaminated materials must be sterilized before discarding.

**This product is for research use only.**