B729	MIDDLEBROOK 7H10 AGAR BASE
Formula	
Ingredients :	**gms/lit.**
Ammonium sulphate	0.50
L-Glutamic acid	0.50
Monopotassium Phosphate	1.50
Disodium phosphate	1.50
Sodium citrate	0.40
Ferric ammonium citrate	0.04
Magnesium sulphate	0.025
Calcium chloride	0.0005
Zinc sulphate	0.001
Copper sulphate	0.001
Pyridoxine hydrochloride	0.001
Biotin	0.0005
Malachite green	0.00025
Agar	15.00
Final pH (at 25°C) : 6.6 ± 0.2	

Directions :

Suspend 9.73 grams in 450 ml distilled water containing 2.5 ml glycerol. Heat to boiling to dissolve the medium completely. Sterilize at 15 lbs pressure (121°C) for 10 minutes. Cool to 45-50°C and aseptically add 50 ml Middlebrook OADC Growth Supplement (BF082). Mix well and pour into sterile screw capped tubes or containers.
Note: Keep prepared medium in the dark before and after inoculation.

Principle :

This medium consist of many inorganic salts which help for the growth of Mycobacteria. Citric acid formed from sodium citrate helps in retaining inorganic cations in solution. Glycerol supplies carbon and energy. Supplement OADC contains oleic acid, bovine albumin, sodium chloride, dextrose and catalase. Oleic acid and other long chain fatty acids are essential for metabolism of Mycobacteria. Dextrose is an energy source. Catalase neutralizes toxic peroxides while albumin protects tubercle bacilli from toxic agents. Malachite green partially inhibits other bacteria.

QC Tests – (I)Dehydrated Medium		
	Colour :	Cream to light green
	Appearance :	Homogeneous Free Flowing powder
(II)Rehydrated medium		
	pH (post autoclaving/heating) :	6.6 ± 0.2
	Colour (post autoclaving/heating) :	Very light amber
	Clarity (post autoclaving/heating) :	Clear to slightly opalescent gel with greenish tinge
(III) Q.C. Test Microbiological		
	Cultural characteristics observed with added Middlebrook OADC Growth Supplement (BF082) and glycerol after an incubation at 35-37°C for 2-4 weeks	
	MICROORGANISM (ATCC)	GROWTH
	Mycobacterium tuberculosis H37 RV (25618)	Good-luxuriant
	Mycobacterium smegmatis (14468)	Good-luxuriant
	Mycobacterium fortuitum (6841)	Good-luxuriant

Precautions :	1. For Laboratory Use.
	2. Follow proper, established laboratory procedures in handling and disposing of infectious materials.

Limitations :	1. Since the nutritional requirements of organisms vary, some strains may be encountered that fail to grow or grow poorly on this medium.				
	2. Negative culture results do not rule out active infection by mycobacteria. Some factors that are responsible for unsuccessful cultures are ; • The specimen was not representative of the infectious material, i.e. saliva instead of sputum. • The mycobacteria were destroyed during digestion and decontamination of the specimen. • Gross contamination interfered with the growth of the mycobacteria. • Proper aerobic conditions and increased CO_2 tension were not provided during incubation.				
Use :	For isolation, cultivation and sensitivity testing of Mycobacterium tuberculosis .				
Storage :	Dehydrated medium- below 30°C Prepared medium– Between 2 to 8°C.				
Packing :	500 gm. bottle				
Product profile:	Reconstitution	Quantity on Preparation (500g)	pH (25°C)	Supplement	Sterilization
B729	19.46 g/l	25.69L	6.6 ± 0.2	Middlebrook OADC Growth Supplement (BF082)	121^0C / 10 minutes