ATCC Medium 1470: Modified Leptospira Medium

REAGENTS:

N/A □ Base Medium

Peptone	0.3 g
Beef Extract	0.2 g
NaCl	0.5 g
Agar (see note section)	1.5 g
Supplement A (see below)	100 mL
Distill water	900 ml

N/A □ Supplement A

Sterile Rabbit Serum	100 mL
*0.05% Hemin solution (see below)	2.5 mL

N/A □ *Hemin solution (0.05%)

Hemin	0.05 g
0.1N NaOH	1 mL
DI Water	99 mL

PROCEDURE:

Steps

N/A To make ATCC Medium 1470:

Accurately weigh out Base Medium reagents **EXCEPT** Supplement A

Dissolve completely into DI water

Start time: End time:

Adjust to pH 7.4.

Autoclave at 121°C for 15 minutes.

Cool to 50°C

Aseptically add sterile supplement A to Base medium (see below for Supplement A)

Adjust final pH to 7.3 ± 0.1

N/A □ To make Supplement A:

Accurately measure Sterile Rabbit Serum and 0.05% Hemin Solution (see below)

Mix well

Filter sterilize

N/A □ To make 0.05% Hemin Solution:

Accurately weigh out Hemin

Dissolve hemin in NaOH

Add DI water.

Autoclave at 121°C for 15 minutes or by filter sterilization

Add to Sterile Rabbit Serum (part of Supplement A)

NOTE:

This media is made with thickened broth. Add 1.5% agar to all vessel types, including bottles and tubes.