

**Document Number: SASoM/METHOD/003.v5****Title: Preparation of media with 5% DCC-FCS for Cell Culture****Version: v5****Author: Peter Mullen**

Effective from:	01/01/2021
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<b>SOP History</b>		
Number	Date	Reason for Change
v1	01/01/2013	Original
v2	01/01/2015	Update
V3	01/01/2017	Update
V4	01/01/2019	Update
V5	01/01/2021	Update

### 1.0 Purpose –

This SOP describes the current procedure for making up cell culture media (DMEM / RPMI) using Dextran-Coated-Charcoal treated Foetal Calf Serum (DCC-FCS) for use in Laboratory 248 at the St Andrews School of Medicine (SASoM). DCC-FCS is used in cell culture experiments where endogenous levels of steroids, hormones or growth factors etc in native FCS may mask the effects of test stimulatory agents such as estradiol, TGF or EGF. Since phenol-red (the pH indicator in normal cell culture media) is known to be weakly estrogenic, media containing NO phenol-red must be utilised. Preparation of Dextran-Coated-Charcoal is carried out as per the relevant SOP.

### 2.0 Scope –

This SOP applies to the staff in the SASoM involved in cell culture work.

### 3.0 Responsibilities –

All staff involved in cell culture are responsible for ensuring that the methods are followed in accordance with this SOP.

All staff must have read and signed the relevant risk assessment documents before performing this procedure.



#### 4.0 Procedure –

Take out 1 x 500mL bottle of phenol-red-free DMEM (Gibco; 11880-028) or phenol-red-free RPMI (Gibco; 32404-014) from the cold room and place in a water bath at 37°C to warm up.

Remove 1 x 27mL aliquot of Dextran-Coated Charcoal-treated Foetal Calf Serum (DCC-FCS), 1 x 10mL aliquot of L-Glutamine and 1 x 5mL aliquot of Penicillin / Streptomycin (Pen/Strep) from the freezer in the cell culture suite. Place them all in the 37°C water bath to defrost. L-Glutamine will remain white and cloudy until it is completely into solution.

Spray the bottle of culture media, the DCC-FCS, the Glutamine and the Pen/ Strep with 70% ETOH and transfer to the hood. Wipe dry.

Under aseptic conditions, add 5mL of L-Glutamine, 5mL of Pen/Strep and 27mL of DCC-FCS to the bottle of media. The bottle of DMEM / RPMI should now have a final concentration of 5% DCC-FCS (27mL FCS in a total volume of  $500+27+5+5=537\text{mL} = 5\%$ ).

Label the bottle along with your name and the date.

Store all made-up media in the fridge or coldroom.

#### 5.0 Personal protection -

A Howie laboratory coat and lab gloves must be worn at all times.

#### 6.0 Spillages -

Always clean up any spills immediately after use, only you know what you have spilt and are aware of its hazard.

Spillages should be mopped up with paper towel, disinfected with 70% ethanol and finally washed with Teknon 100.

#### 7.0 Training -

All staff should be trained in sterile TC techniques before starting any TC work

#### 8.0 Related documents –

- 8.1 Risk assessments – COSHH /004 and RA/BIOL/004
- 8.2 SOP - SASoM/METHOD/004  
Dextran-Coated-Charcoal treatment of FCS (DCC-FCS)
- 8.3 SOP - SASoM/METHOD/007  
Aliquoting TC supplements



## 9.0 Approval and sign off –

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Signature: \_\_\_\_\_ Date: \_\_\_\_\_

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