Document Number:	**SASoM/METHOD/004.v5**
Title:	**Dextran-Coated-Charcoal Treatment of FCS (DCC-FCS)**
Version:	**v5**
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Effective from:	01/01/2021
Valid to:	01/01/2023

SOP History		
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 preparing Dextran-Coated-Charcoal Foetal Calf Serum (DCC-FCS) 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. These endogenous steroids are stripped from FCS using dextran-coated charcoal and type IV sulphatase (EC 3.1.6.1.).

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 –
Remove 1L (2 x 500ml) of FCS from freezer and thaw overnight at 4°C.

Transfer FCS to a pre-warmed water bath at 37°C for 15 min.

Heat-inactivate the FCS by placing in a separate water bath (filled to a level above the FCS in the bottles) at 56°C for 30 min, inverting the bottles three times after 15 mins to ensure contents have not settled.

For 1L of serum, add 2000 Units of type IV sulphatase and incubate for 2 hours at 37°C. *(Sigma S9626; current stock is 15000 units/g)*

Adjust pH to 4.2 using 2M HCl.

Add a pre-prepared charcoal mix (5g charcoal and 25mg Dextran T70 in 50ml distilled H_2O per 1L of FCS). Cover in foil and stir overnight in cold room.
Charcoal; Sigma C5120 & Dextran T70: Sigma 31390.

Separate charcoal from the FCS by centrifugation at 10,000rpm using a Sorval RC6 centrifuge and GSA-1500 rotor (30 min @ 4°C) with suitable tubes.

Gently and smoothly pour the FCS into a large beaker, leaving behind as much of the charcoal as possible. Re-adjust pH to 4.2 as above.

Add a second pre-prepared charcoal mix (5g charcoal and 25mg Dextran T70 in 50ml H_2O per 1L of FCS). Cover in foil and stir overnight in cold room.

Remove charcoal from the FCS by centrifugation at 10,000rpm using Sorval RC6 centrifuge and GSA-1500 rotor (30 min @ 4°C) as described above.

To remove remaining residual charcoal traces, repeat previous centrifugation step at 10,000rpm using Sorval RC6 centrifuge and GSA-1500 rotor (30 min @ 4°C).

Return FCS to pH 7.2 using 2M NaOH.

Filter sterilise FCS using Stericup filters (Sterilin-Bibby: SCGV U05 RE).

Aliquot out into sterile 50ml disposable tubes (27ml per tube) and label as DCC-FCS with the date.

Store aliquots at -20°C.

5.0 Personal protection –
A Howie coat must be worn at all times. Gloves as specified in the appropriate COSHH RA.

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 detergent.

7.0 Training -

All staff should complete an in-house induction to the tissue culture area and 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/003
Making up DMEM / RPMI Media containing 5% DCC-FCS for Cell Culture

8.3 SOP SASoM/METHOD/007
Aliquoting TC supplements

9.0 References –

The method was adapted from: *Stanley, E.R., Palmer, R.E. and Sohn, U. (1997). Development of methods for the quantitative in vitro analysis of androgen-dependent and autonomous Schionogi carcinoma 115 cells. Cell. 10:35-44.*

10.0 Approval and sign off –

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