Document Number:	**SASoM/METHOD/016.v5**
Title:	**Preparation of ß-Estradiol (1x10^{-2}M) stock solution**
Version:	**v5**
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SOP History		
Number	Date	Reason for Change
v1	01/01/2013	General
v2	01/01/2015	Update
V3	01/01/2017	Update
V4	01/01/2019	Update
V5	01/01/2021	Update

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1.0 Purpose –

This SOP describes the current procedure for preparation of a ß-Estradiol stock solution in Laboratory 248 at the St Andrews School of Medicine (SASoM).

The concentration of ß-Estradiol used depends partly on the question being asked in that if we are doing estrogen-only type experiments, we may get a slightly better response with 10^{-9}M than 10^{-10}M. However, if the study involves using an anti-estogen such as Tamoxifen, then we have 10X more estrogen to oppose and therefore we have generally gone for 10^{-10}M ß-Estradiol together with 10^{-6}M Tamoxifen.

The conventional wisdom is that you need between 1,000 - 10,000x more Tamoxifen than ß-Estradiol in order to achieve a guaranteed block.

2.0 Scope –

This SOP applies to the staff in Laboratory 248 at the St Andrews School of Medicine (SASoM) involved with preparation of ß-Estradiol stock solution.

3.0 Responsibilities –

All staff involved in stock preparation 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 –

ß-Estradiol is purchased in powder form (Sigma; E8875) and stored in a cytotoxic cabinet at room temperature.

The molecular weight of ß-Estradiol is 272.4kDa.

ß-Estradiol should be made up as a $1x10^{-2}$M stock solution and subsequently stored at -20°C in a suitable freezer.

Weigh out 27.2mg of ß-Estradiol into a sterile glass vial (1) and add 10ml of absolute Ethanol. Mix thoroughly and make sure that the powder has all gone into solution.

Protect from light by covering the vial in Tin-Foil.

Label as '$1x10^{-2}$M ß-Estradiol' along with your initials, the date and an expiry date of 3 months (2).

Place inside an airtight container and store upright in a suitable freezer at -20°C. Make sure the box is labeled with appropriate hazard labels.

Use at a final concentration of $1x10^{-9}$M by making serial dilutions as detailed below:
$1x10^{-5}$M = 1:1000 dilution of $1x10^{-2}$M stock (10µl/10ml) in ETOH.
$1x10^{-7}$M = 1:100 dilution of $1x10^{-5}$M solution (100µl/10ml) in cell culture media.
$1x10^{-9}$M = 1:100 dilution of $1x10^{-7}$M solution (100µl/10ml) in cell culture media.
Or
$1x10^{-10}$M = 1:1000 dilution of $1x10^{-7}$M solution (10µl/10ml) in cell culture media.

N.B.
(1) ß-Estradiol Stock Solution must be made up in a glass vial although further serial dilutions can be made up in plastic universal containers.
(2) ß-Estradiol Stock Solution should be made up fresh every 3 months.

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.

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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

9.0 Approval and sign off –

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