Document Number:	**SASoM/METHOD/012.v5**
Title:	**Preparation of Heregulin-B for Cell Culture**
Version:	**v5**
Author:	**Peter Mullen**

Effective from:	01/01/2021
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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 sterile aliquots of Heregulin-ß for cell culture use in Laboratory 248 at the St Andrews School of Medicine (SASoM). Since considerable variations have been seen from different manufacturers, the supplier and product number (R&D #396-HB/CF) should not be changed without good cause!

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 Heregulin-ß (R&D #396-HB/CF) from -20ºC freezer and transfer to ice - keep on ice at all times. Place 65 x 0.6ml sterile microcentrifuge tubes on ice and allow to chill.

Under aseptic conditions in the cell culture hood, add 0.5ml Sterile PBS to a 50µg vial of Heregulin-ß.

Gently mix by inverting tube a number of times until the contents have gone into solution.

Slowly add a further 0.5ml of sterile PBS (total volume = 1.0ml).

Gently mix by inverting tube (x10).

Aliquot out 65 x 15µl aliquots into sterile 0.6ml microcentrifuge tubes, keeping on ice throughout.

Store aliquots as a batch in a -20ºC freezer.

Use at a final concentration of $1x10^{-9}$M (1nM) by adding 6µl/10ml of culture media containing Dextran-Coated-Charcoal FCS (DCC-FCS).

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/METGHOD//014
Making up PBS for Cell Culture

9.0 Approval and sign off –

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