Document Number:	**SASoM/METHOD/018.v5**
Title:	**Flow Cytometric DNA Analysis of Cell Lines**
Version:	**v5**
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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 samples for flow cytometric DNA & ploidy analysis of cell lines in Laboratory 248 at the St Andrews School of Medicine (SASoM). The method is an adaption of Vindeløv et al (Cytometry 1983; 3(5): 323-327).

2.0 Scope –
This SOP applies to the staff in the SASoM involved in Flow Cytometric DNA Analysis of Cell Lines.

3.0 Responsibilities –
All staff involved in the Flow Cytometric DNA Analysis of Cell Lines 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 –
Citrate Buffer:
Dissolve 85.5g Sucrose (S9378, Sigma) and 11.76g of Trisodium Citrate (301287F, BDH) in 800ml distilled water. Add 50ml DMSO (140214P, BDH) and adjust to pH7.6

(with 2-3 drops of concentrated HCl). Make up to 1000ml with distilled water and store at 4ºC.

Stock Solution:
Dissolve 2000mg Trisodium Citrate (301287F, BDH), 121mg Tris Base (T1378, Sigma), 1044mg Spermine Tetrahydrochloride (S2876, Sigma) and 2ml Nonidet NP40 (N3516, Sigma) in 1800ml distilled water. Adjust to pH7.6 and make up to 2000ml with distilled water.

Solution A:
Dissolve 15mg Trypsin Type IX-S (T0303, Sigma) in 500ml Stock Solution pH7.6, dispense into 25 x 20ml aliquots, label and freeze at -20ºC.

Solution B:
Dissolve 250mg Trypsin Inhibitor (T9253, Sigma) and 50mg RNAse A (R4875, Sigma) in 500ml Stock Solution pH7.6, dispense into 25 x 20ml aliquots, label and freeze at -20ºC.

Solution C:
Dissolve 208mg Propidium Iodide (81845, Sigma) and 500mg Spermine Tetrahydrochloride (S2876, Sigma) in 500ml Stock Solution pH7.6, dispense into 25 x 20ml aliquots, label and freeze at -20ºC.

Method:
Trypsinise cells in the usual manner and transfer to a 5ml (12 x 75mm) 'BD Falcon' tube (352052; BD Biosciences). Centrifuge at 1600rpm for 5mins. Invert tubes and allow excess moisture to be absorbed onto a paper towel. Resuspend cells in citrate buffer (100µl), cover tubes with parafilm and store at -20ºC prior to analysis.

Defrost Solutions A, B and C (keeping solution C on ice after thawing).

Defrost samples at room temperature.

(Add Chicken Red Blood cells (CRBC), Trout Red Blood cells (TRBC) or human lymphocytes as internal DNA ploidy standards if required).

Add 450µL Solution A, vortex and incubate at room temperature for 2 min (mixing throughout the incubation).

Add 375µL Solution B, briefly votex and incubate at room temperature for a further 10 min.

Add 250µL Solution C, briefly vortex and incubate on ice in the dark for a further 10 min. Keep on ice and in the dark prior to analysis.

Run samples using the Flow Cytometer according to the appropriate settings, templates, etc.

Cell cycle analysis can be performed using FlowJo7 software.

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 under go training in this technique before performing the procedure.

8.0 Related documents –

8.1 Risk assessments COSHH/014
RA/BIO/004

8.2 SOP SASoM/METHOD/008
Use of Trypsin for passaging cells in Cell Culture.

9.0 Approval and sign off –

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