Generation of EBV-immortalized B cell lines

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Changes from last version			
Date of version	**Paragraphs**	**Date of version**	**Paragraphs**

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Content

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1. Subject

This Standard Operation Procedure (SOP) describes a procedure to immortalize B cell lines using Epstein-Barr Virus (EBV).

2. Application

Immortalization of B cells by EBV is an effective procedure for inducing long term growth of certain human B lymphocytes.

3. Definitions, Terms and Abbreviations

PBMC = Peripheral Blood Mononuclear Cells
EBV = Epstein-Barr virus
FCS = Foetal Calf Serum
RPMI = Roswell Park Memorial Institute
P/S/G = Penicillin-Streptomycin L-Glutamine
FACS= Fluorescence Activated Cell Sorter
v/v = Volume / volume
w/v = Weight/ volume
ml = Millilitre

4. Principle

Epstein-Barr virus is the only virus known to immortalize human B lymphocytes. It transforms human B lymphocytes to indefinitely proliferating cells in tissue culture. These cells are initially polyclonal and secrete all major classes of immunoglobulin, but after a prolonged culture *in vitro*, the cell lines may become oligoclonal or monoclonal.
B cell immortalization by EBV in culture will be suppressed when T cells from EBV seropositive persons are also in the culture. Therefore, immortalization of B lymphocytes by EBV occurs with greater frequency if T cells are either physically removed from the culture or are functionally inactivated with for example cyclosporin A.

5. Safety Precautions

Treat every sample containing human material as infectious material, wear disposable gloves.

Epstein-Barr Virus
(B95-8 cell line)

Biohazard
Risk group 2

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Ethanol	F, Flammable	
Cyclosporin A	 Cyclosporin A Xi, Irritant	Xi, Irritant

6. Reagents

6 .1 Chemicals

Reagents	Formula	Supplier	Order number	Store at
Cyclosporin A	-	Novartis	S0039	4°C
RPMI 1640	-	Invitrogen	52400-025	4°C
L-glutamine	-	Invitrogen	25030-024	-20°C
Penicillin-Streptomycin	-	Invitrogen	15140-122	-20°C
Foetal Bovine Serum	-	Invitrogen	10270-106	-20°C
EBV supernatant B95-8 cell line (filtered with 10% FCS)	-	ATCC		
Ethanol	CH_3OH	Pharmacy (WKZ)		RT

6.2 Culture Medium (RPMI with P/S/G and 10% FCS)

RPMI 1640 supplemented with 1 v/v% P/S (= 5ml), 1 v/v% glu (=5ml) and 10% v/v FCS (=50 ml)
FCS should be heat inactivated (1 hr at 56°C) and filtered through a 0.20 µm sterile filter using a 10 ml sterile syringe before usage. Store at 4°C up to 1 month.

7. Equipment and Accessories tools

7.1 Equipment

- Centiguge Hettich Rotanta 46 (UMC# 99-000-2142)
- Reichert brightline hemacytometer (Hausser Scientific Company, Horsham PA, USA)
- Easypet pipet (Eppendorf, Germany, 4006173)
- Pipets 10-1000 µl (Gilson, The Hague, The Netherlands)
- Waterbath 37°C (GFL, Burgwedel Germany, UMC# 45-12-02-00-007)
- Incubator, 37°C and 5% CO_2

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7.2 Accessories

- 50 ml sterile polypropylene conical tubes (Falcon/ Becton Dickinson, Erembodegem, Belgium, 352070)
- Sterile 10 ml syringe (Becton Dickinson, Erembodegem, Belgium)
- Sterile 50ml syringe (Tyco Healthcare, Gosport, Northern Ireland, 1100 660131)
- Minisart 0,20µm single use sterile filter (Sartorius, Hanover, Germany, 16534)
- 2 ml sterile disposable pipet (Falcon/ Becton Dickinson, Erembodegem, Belgium, 357507)
- 5 ml sterile disposable pipet (Falcon/ Becton Dickinson, Erembodegem, Belgium, 357543)
- 10 ml sterile disposable pipet (Falcon/ Becton Dickinson, Erembodegem, Belgium, 357551)
- 25 ml sterile disposable pipet (Falcon/ Becton Dickinson, Erembodegem, Belgium, 357525)
- Disposable gloves (Kimberly Clark, Zaventem, Belgium)
- 96-Wells round bottom sterile culture plate (Nunc Roskilde, Cat.No 163320)
- Easyload 200µl (741035) and 1000µl (741000) sterile pipettips (Greiner Bio one, Germany)
- Falcon 5ml Polystyrene Round-bottom tube (Becton Dickinson, Cat.No. 352058)

8. Samples

8.1 Sample collection

(Preferably) fresh PBMC (see UCAN-U 0001)

8.2 Sample Processing

All handlings of the sample should be done in a biohazard safety cabinet

9. Procedure

1. Isolate PBMC using Ficoll method (see UCAN-U 0001)
2. Resuspend 2 x 10^6 cells in 0,25ml 10% FCS culture medium in a 5ml FACS tube.
3. Add 0,25ml EBV supernatant from the B95-8 cell line and incubate for 2 hours in the water bath on 37°C.
4. Add 0,5ml 10% FCS culture medium and 1µl cyclosporin A. (Final concentration 1µg/ml)
5. Transfer the cells to a 96-wells plate (200µl/well; 5wells) and put in the incubator 37°C and 5% CO_2.
6. Split wells when medium becomes yellow and/or 'satellites' form. Until then, refresh medium weekly by taking off 100µl 10% FCS medium and adding 100µl fresh FCS medium.

10. Literature

1. Tosato, G. and Cohen, J.I. 2007. Generation of Epstein-Barr Virus (EBV)-Immortalized B Cell Lines. Current Protocols in Immunology 76:7.22.1-7.22.4
2. Sugden, B. and Mark, W. 1977. Clonal Transformation of Adult Human Leukocytes by Epstein-Barr Virus. Journal of Virology, 23(3): 503-508
3. Áman, P., Ehlin-Henriksson, B. and Klein, G. 1984. Epstein-Barr Virus susceptibility of normal human B lymphocyte populations. J. Exp. MED, 159(1): 208-220
4. Miller, G. 1982. Immortalization of Human Lymphocytes by Epstein-Barr Virus. Yale J Biol Med. 55(3-4): 305-310

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11. **Remarks**

- Isolated PBMC should be from a typed MHC class II homozygote healthy donor.
- The B lymphocytes should be viable, of sufficient number and resting.
- An adequate number of infectious EBV particles should be added to the cells to be immortalized. B95-8 culture supernatant containing EBV should not be removed after B lymphocyte infection for at least the initial 3 week period.
- Cyclosporin A should be present in the medium during the first 2 - 3 weeks of culture.

***** End *****