HVS Transformation of Human PBMC's

Date: Wed, 7 Jan 1998 17:20:44 -0500
From: jjung@warren.med.harvard.edu (Jae Jung)
Subject: Re: HVS
To: Erle Robertson <esrobert@umich.edu>

Dear erle,
happy new year. have you sent the same mail before? that means i did not reply back. sorry about that. life is still hectic here. papers, renewal and other stuffs! it is good to hear that things are moving now. since we are making il-2 independent T cell transformation, protocol we are using is very simple.
Primary peripheral blood mononuclear cells (PBMCs) from common marmosets Callithrix jacchus) were purified from 3 ml heparinized blood specimens using lymphocyte separation medium (Organon Teknika Corp., Malvern, Pa.). Washed cells were resuspended and cultured in RPMI 1640 medium supplemented with 20 % (vol/vol) heat inactivated fetal bovine serum and 5 µg/ml of beta -mercaptoethanol. HVS infection of marmoset PBMCs was performed in 12 well tissue culture plates by addition of 1ml of HVS. Immortalization of primary lymphocytes to IL-2 independent growth was typically established within a month after infection. Uninfected common marmoset PBMCs were also cultivated by stimulation with 1 µg of phytohemagglutinin per ml for 48 hrs followed by culture in RPMI 1640 with 20% fetal bovine serum and 10% IL-2.
good luck with your experiments.

Conc. BME 14.3M
= 1.1g/ml
5µl/ml = 5/9 g/ml
Add 500µl/500ml Med
RPMI

best regards
jj

_____ Reply Separator _____

Subject: HVS
Author: Erle Robertson <esrobert@umich.edu> at HMS-Internet
Date: 1/7/98 11:51 AM

Dear Jae,
Happy New Year. I hope that the research is going well and that you are having a wonderful time with your family and friends. I am doing fine and the lab is just begining to look like experiments are getting done the way I would like to see things moving.

I am trying to do some transformation assays with HVS which you supplied and I am having trouble. I was wondering if you can supply me with a protocol you have used in the past for doing these assays. I would be very grateful if you would assist in this manner. Fleckenstein group does not wnat to give up the cosmids for HVS just yet. I am in the process of doing the cloning myself and I hope that everything goes well. I am presently making a vector so that I can take out unique fragments as they were cloned.

I look forward to hearing from you,

Best and sincere regards,
Erle

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