Date: Tue, 15 Aug 2000 19:28:02 -0400 (EDT)
From: "Robert A. Thomas" <rathomas@cmb.biosci.wayne.edu>
To: Erle Robertson <esrobert@umich.edu>
Subject: Re: Hello

Here is the recipe and protocol that you requested:

THE CELL LYSIS BUFFER IS COMPRISED OF TWO BUFFERS:

BASIC CELL LYSIS BUFFER The Basic buffer is made as a 5X stock which is autoclaved

15 mM CaCl2 (MW 111.0)	0.167g/100 ml
10 mM Magnesium acetate (MW 214.46)	0.215g/100 ml
0.5 mM EDTA (w/ Na2 MW 372.2)	0.019g/100 ml
50 mM TRIS-HCl [pH 8.0] (MW 121.14)	0.606g/100 ml

COMPLETE CELL LYSIS BUFFER is made as follows:

1X BASIC BUFFER (See above)	20 ml /100 ml
1 mM DTT (MW 154.2)	0.154g /100ml
0.5% v/v NP40	0.5 ml/100 ml

CELL LYSIS PROTOCOL

1) Transfer cells suspension into 15 centrifuge tube and gently pellet cells.
2) Wash cells 2X with 2X DPBS (save supernatants)
3) Resuspend the pellets in 125 ul of COMPLETE CELL LYSIS BUFFER.
4) Incubate 20' at room temperature with vortexing
5) Store cell lysates at -80 until ready for assay (PCR, luciferase, CAT)

If you have any questions, let me know.

Bob

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On Tue, 15 Aug 2000, Erle Robertson wrote:

> Hi Robert,
> What's going on buddy. I tried calling you a couple of times with no response. My student will do the transfection tomorrow inbothe th 8E5 and ACH2 cell lines and we need to know what the protocol for preparing the cells for PCR analysis of the HIV transcripts from the LTR. Also we need some p24 and Tat antibodies for western blot to correlate these studies with the RNA.
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> Thanks and I look forward to hearing from you again,
> Erle
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