

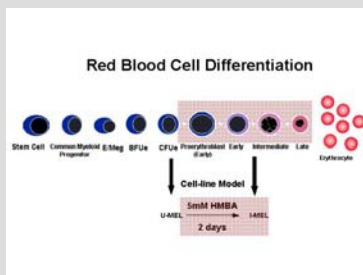
# High Content Screening of Red Blood Cell Differentiation and Cytotoxicity Using an Acumen Explorer

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## Abstract

$\beta$ -thalassaemia is caused by mutations in the  $\beta$  globin genes which result in reduced  $\beta$  globin chain synthesis in mature red blood cells. The major pathophysiology, however, is the resulting excess of  $\alpha$  globin chains, which precipitate and cause oxidative damage in the cells. This study intends to find agents of therapeutic potential which lower  $\alpha$  globin gene expression, therefore reducing the need for blood transfusions in patients with severe forms of the disease. A multiplex cell based assay using an Acumen Explorer fluorescence microplate cytometer was developed to quantitate both  $\alpha$  globin expression and cell cytotoxicity during terminal differentiation of red blood cells. Mouse erythroleukaemia (MEL) stable cell lines which contain the human  $\alpha$  globin promoter driving an eGFP reporter were used to measure  $\alpha$  globin expression after hexamethylene-bisacetamide-induced (HMBA) terminal differentiation. Use of the high content assay for compound screening is presented.

## Red Blood Cell Differentiation



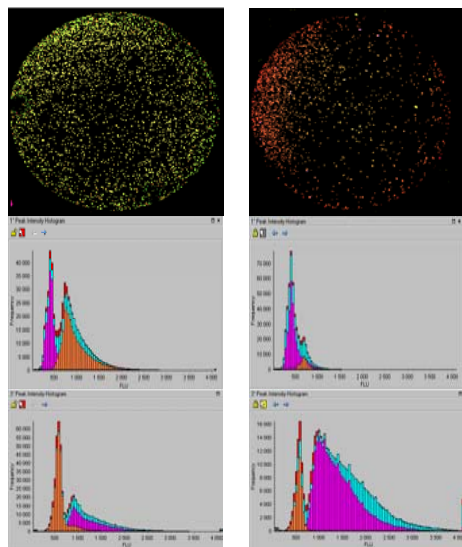
MEL provide a model for late stage differentiation of red blood cells from myeloid progenitor cells. In the uninduced state (MEL-U) there is no globin gene expression. Induction with 5 mM HMBA for two days triggers terminal differentiation and globin gene expression is activated in induced cells (I-MEL).

MEL stable cell lines were developed containing a construct with the human alpha-globin promoter driving GFP expression under the control of the major upstream regulatory element (HS-40) of alpha-globin gene expression. Treatment with HMBA induces terminal differentiation and induction of the reporter expression is observed. Clones were selected with high levels of induced FP expression, which could be detected on either a fluorescence plate reader or the Acumen Explorer.

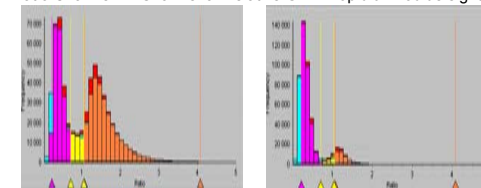
MEL cells are non-adherent, and as such are not amenable to heterogeneous assay formats for HTS. An homogeneous screen to quantitate both GFP expression and cytotoxicity was required.

## 1 MEL GFP-globin gene induction

5 mM HMBA 2 days      Uninduced



Ratio Channel 1 / Channel 3      Relative GFP/Propidium Iodide signals



### Acumen Explorer Parameters Quantified

Red: Fluorescent Objects detected in Channel 1 or Channel 3.  
 Turquoise: Cells defined by size width 4-100 $\mu$ m, depth 4-100 $\mu$ m.  
 Pink: Dead cells stained Propidium Iodide: Ratio Channel 1:Channel3 <0.2 GFP:PI<0.7  
 Yellow: Dying Cells with both PI and GFP associated with them ratio 0.71<GFP:PI<1.0  
 Orange: GFP expressing cells GFP:PI>1.1

### Acumen Explorer Parameters Reported

Objects: Total number of cells, Total GFP living, Dying and Dead cells  
 Area: Total area GFP cells  
 Fluorescence: Total fluorescence intensity GFP cells

## 2 Assay Protocol

### Induction

Day 1 Plate cells 10,000 per well in media, 1% serum, 5 mM HMBA into 96-well black plates clear bottom 200  $\mu$ L per well. Treat with compound or cycloheximide control

Day 3 Add Propidium Iodide to well and read on an Acumen Explorer.

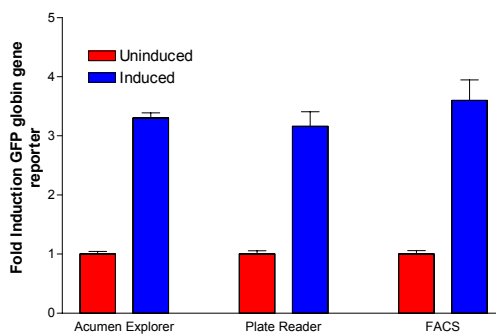
### Cytotoxicity

Assay Propidium Iodide is impermeable to live cells. Fluorescent staining of DNA in the nuclei of dead or dying cells with damaged membranes only are detected.

Calculate Percent Total Cell GFP Fluorescence per well relative to untreated control. Percent PI-stained cells per well.

Throughput 10 minutes/plate. Single read.

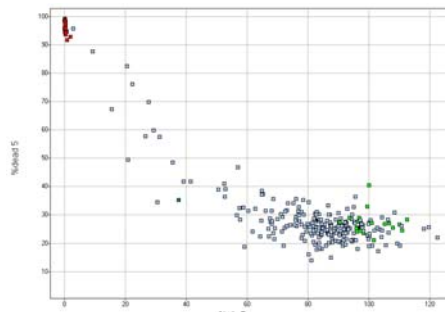
## 3 GFP Reporter Induction



## Conclusion

- The Acumen Explorer enabled the development of an homogeneous assay for non-adherent cells which combined reporter expression and cytotoxicity data in a single read.
- The sensitivity of the GFP signal observed on the Acumen Explorer was greater than that using the plate reader and was comparable to that obtained with a luciferase reporter.
- Robustness was demonstrated using a test set of 1,000 compounds. A further 10,000 have been screened and hits are being evaluated.

## 4 Test Screen Data



Cells were incubated with 5 mM HMBA, 1% serum, IMDM for two days in the presence of 5  $\mu$ M compound (blue), or vehicle control (green) - 0.5% DMSO. Cycloheximide control shown in red.

Test Screen: 1000 purified natural products  
 Hit Rate (<50% GFP): 3.9% (90% of which were toxic)  
 Confirmation Rate: 50%

## 5 Comparison of Assay Performance

	Reader -GFP (RFU)	Reader -Luc (RLU)	Acumen (Total cell RFU)
Cells per well	100,000	5-20,000	5-20,000
HMBA	17818±635	15989±464	21±1.5 X10 <sup>6</sup>
Cycloheximide	11044±161	2323±484	2±2 X10 <sup>5</sup>
S.B	1.6	31.6	100
S.N	10.3	13.5	14
Z	0.65	0.77	0.78