USING TURNER BIOSYSTEMS INSTRUMENTS FOR THE SECRETED ALKALINE PHOSPHATASE ASSAY

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INTRODUCTION

Quantitation of secreted alkaline phosphatase (SEAP) has become a powerful tool for investigating promoter activity in transfected eukaryotic cells [1]. SEAP assays have been used for a large variety of applications including promoter and enhancer studies, gene expression analysis, gene knockdown/RNA interference read-out studies, viral functional assays, and in vivo reporter gene assays. The SEAP reporter gene encodes a truncated form of the human placental alkaline phosphatase gene that lacks the membrane anchoring domain. Therefore, the protein can be efficiently secreted from transfected cells allowing for detection of reporter gene activity without cell lysis [1, 2]. Using a secreted reporter protein has several advantages over traditional reporter assays: 1) Cell lysis is not required for analysis so a single set of cells can be used for both the SEAP assay and another purpose, 2) Gene expression kinetics can be studied by the repeated collection of the culture medium from the same cultures, 3) By changing the culture medium prior to an experiment, the assay background is reduced to almost zero.

SEAP assays utilize enzyme activity of alkaline phosphatase to dephosphorylate the chemiluminescent alkaline phosphatase substrate CSPD™ (PubChem CID No. 424756) into an unstable dioxetane anion which decomposes and emits light. The additional use of chemiluminescent enhancing reagents can increase the light output up to 500 times. Because of the reduced background signal, the SEAP reporter gene products are among the most sensitive enzymatic reporters available and have a linear range of 4 to 5 logs. SEAP assays can be performed in both single tube and microplate formats using kits available from several manufacturers including Clontech, Roche, and Applied Biosystems (Life Technologies). Turner BioSystems offers a range of instruments that are compatible with the SEAP assay. The Modulus™ II Microplate, Modulus™ Microplate, Veritas™ Microplate Luminometer, $20/20^n$ Single Tube Luminometer, and Modulus™ Single Tube instruments easily meet the sensitivity and dynamic range requirements of the SEAP assay.

Boivin *et al* illustrate the use of the SEAP assay, combined with a Turner BioSystems Veritas™ microplate luminometer, in their investigations of glucocorticoid (GC) regulation of the intestinal tight junction barrier during TNF-α-induced inflammation [3]. Tight junction permeability is an important risk factor for Crohn's disease, a chronic inflammatory bowel disease. Using the Clontech Great EscAPe™ SEAP assay kit and the Veritas™ Luminometer, the authors were able to demonstrate that Prednisolone, a GC used to treat Crohn's disease, caused an upregulation of GC-receptor responsive promoter. The upregulation of the GC-receptor responsive promoter was further shown to lead to inhibition of myosin light chain kinase (MLCK), a protein previously shown to play a central role in regulation of intestinal tight junction permeability. This experiment, along with other data presented in the paper, helped to better illustrate the mechanism of glucocorticoid mediated tight junction regulation during active inflammation. Because tight junction permeability is associated with both active Crohn's disease and risk of recurrence after treatment, understanding the mechanism of GC mediated tight junction regulation could lead to better treatments and improved accuracy in predicting treatment response in Crohn's disease patients.

SEAP ASSAY PROCEDURES

Regardless of the assay kit used, the SEAP assay is fairly simple to run and consists of the following steps:

1. Remove media from cultured cells that have been transfected with the SEAP reporter vector
2. Dilute assay regents to appropriate concentrations and allow to equilibrate to room temperature
3. Dilute samples with dilution buffer and incubate diluted samples for 30 min at 65°C
4. Add Assay Buffer to each sample and incubate for 5 min at room temperature
5. Add Substrate Reagent and incubate for 10 - 20 minutes at room temperature
6. Measure light output using either a single tube or microplate luminometer with a .1 to 5 second integration time

Once Substrate Reagent has been added to the samples, the light output reaches a stable level after 10 minutes and is stable for up to 1 hour. The dilution buffer contains inhibitors that target non-placental alkaline phosphatase from both serum and endogenous alkaline phosphatase. The combination of inhibitors and heat inactivation step eliminates non-placental alkaline phosphatase activity. However, certain cell lines, such as HeLa and others derived from cervical cancers, may express placental alkaline phosphatase which may produce high assay background and are not recommended for use with SEAP systems.

SEAP kits are also available for dual reporter assays such as the Clontech Ready-To-Glow™ Dual Secreted Reporter System which combines SEAP reporter activity with an expression vector encoding a truncated luciferase enzyme from the marine copepod *Metridia longa*. These kits can be used to monitor two promoters simultaneously or to control for transfection efficiency. Like the single reporter SEAP assay, this dual reporter assay does not require cell lysis, leaving the cells viable for further studies.

CONCLUSIONS

Boivin *et al* demonstrate the use of the Turner BioSystems Veritas Microplate Luminometer with the SEAP assay. With wide dynamic range and superior sensitivity, the Turner BioSystems line of luminometer instruments, Modulus™ II Microplate, Modulus™ Microplate, Veritas™ Microplate, $20/20^n$ Single Tube and Modulus™ Single Tube are ideally suited to match the sensitivity requirements of the SEAP assay. Combined with a Turner BioSystems instrument, the SEAP assay is a powerful tool for researchers who want to perform live cell assays without sacrificing sensitivity.

REFERENCES

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TRADEMARKS

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