The Secreted Alkaline Phosphatase (SEAP) Reporter System

BACKGROUND:

SEAP is a secreted form of embryonic alkaline phosphatase, which is a truncated form of human placental alkaline phosphatase (PLAP) through the deletion of a GPI anchor. Unlike intracellular reporters, SEAP is secreted into the cell culture supernatant, allowing for easy detection without adverse effects on the cell. Other advantages include that it is very heat-stable, resistant to inhibitor L-homoarginine, and can be used for kinetic studies.

Original InvivoGen Product Info:

SEAP Reporter Gene System	http://www.invivogen.com/seap-reporter-gene-system
HEK Blue Detection Media	http://www.invivogen.com/hek-blue-detection
pNIFty2-SEAP Plasmid	http://www.invivogen.com/pnifty2-seap

The pNIFty2-SEAP plasmid is composed:

1) an ELAM proximal promoter
2) 5 NF-κB repeated transcription factor binding sites (TFBS)
3) SEAP Reporter Gene

Other Details: Selectable with Zeocin in mammalian cells and bacteria & can be used to generate stable cell lines. The plasmid can be used to transfect TLR transfected cells.

MECHANISM OF ACTION:

Upon recognition of a PAMP, receptor signaling (*i.e.* TLR, Nod1/2 receptor) leads to the downstream activation and translocation of NFκB, a transcription factor that induces a pro-inflammatory response. In these cells, activation of NFκB leads to the expression of SEAP via the ELAM proximal promoter, which can be detected through the use of HEK Blue Detection Media (Invivogen).

USING THE SEAP REPORTER SYSTEM:

The pNifty2-SEAP plasmid (Invivogen) can be either transiently or stably transfected into mammalian cells, which express TLRs. If the cell line does not express any TLRs (or other receptors that would induce NF-κB activation), these receptors can be transfected concurrently with the reporter plasmid.

Note: creating stable lines expressing your receptor of interest & the SEAP-NF-κB reporter plasmid will increase efficiency, however, transient transfections may work better for some lines.

Following transfection, wells may be stimulated with ligands or samples by adding it them directly to the wells, diluted in HEK Blue Detection (HBD) Media. Note that ligands may also be transfected in. HBD media serves as a fast and convenient method to monitor SEAP expression in a time-dependent manner. In the presence of alkaline phosphatase activity, the media changes in colour to a purple or blue hue, which can be quantified via absorbance spectroscopy at 620-655nm. The colour change can usually

be observed by the naked eye.

• You may use a recombinant SEAP protein as a positive control, which also can be attained from Invivogen.
• Another method of SEAP Detection would be the QUANTI-Blue detection assay

HOW DO I USE IT?

I use both a cell line of HEK29Ts, stably expressing mNod2 & SEAP-NF-κB, in addition to a HEK293/MD2-CD14-TLR4 line that is transiently transfected with the SEAP-NF-κB reporter plasmid.

Basic Transient Transfection Protocol using PEI:

The day prior to transfection, plate $1x10^4$ cells per well into a 24-well plate in Complete DMEM. Depending on the cell line, the number of cells may differ.

1. For each 24-well plate to be transfected, mix 25ul serum free media and 450ng of your DNA of interest into a polystyrene tube.
The amount of DNA to be used is dependent on the type of DNA. For transfections with hNod1/hNod2, 5ng of the total 450ng was either hNod1 or hNod2 and the remainder was an empty pcDNA plasmid.

2. If cell line does not consist of a reporter, add 50ng of reporter per well to solution.
A SEAP-NFkB reporter worked well for these transfections.

3. Add 3ul of PEI (per well). Immediately pulse vortex for 10 seconds.

4. Allow solutions to incubate at room temperature for 10 minutes. In the meantime, discard old media from 24-well plates.

5. After incubation time, 150ul per well of serum free media was added to the solution.
It might be easier to add the 150ul of media directly to the wells of the plate rather than to each solution.

6. Add each PEI solution in a drop-wise manner to the corresponding cells. Make sure that drops are distributed over entire well. Gently rock the plate to ensure even distribution. Do not swirl plate.

7. Incubate the plate(s) in the 37°C, CO2 Incubator. The duration required for transfection to occur depends on the cell line and the DNA being transfected.
For transfection of hNod1 or hNod2 DNA into HEK293T cells, it takes between 48-72 hours

8. After 3 hours of incubation, add 1mL of 1% FBS DMEM to each well.
If a SEAP-NFkB reporter was also transfected, you may add 1mL of HEK Blue Detection Media (Invivogen) instead of the 1% FBS DMEM. This will allow for real-time detection of SEAP expression. HEK Blue Detection Media may also be added at time of ligand addition.