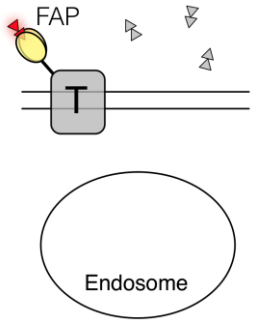
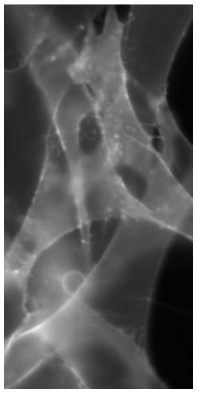
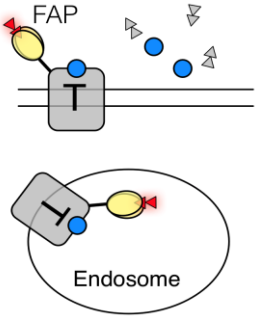
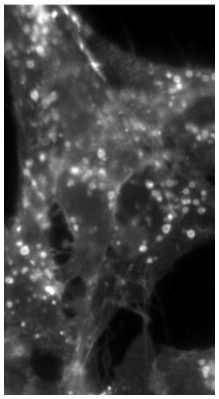
www.spectragenetics.com 412-488-9350 sales@spectragenetics.com

ADRB2 Internalization Assay By Surface Fluorescence Depletion or Internal Fluorescence Accumulation Using FAP®-tags

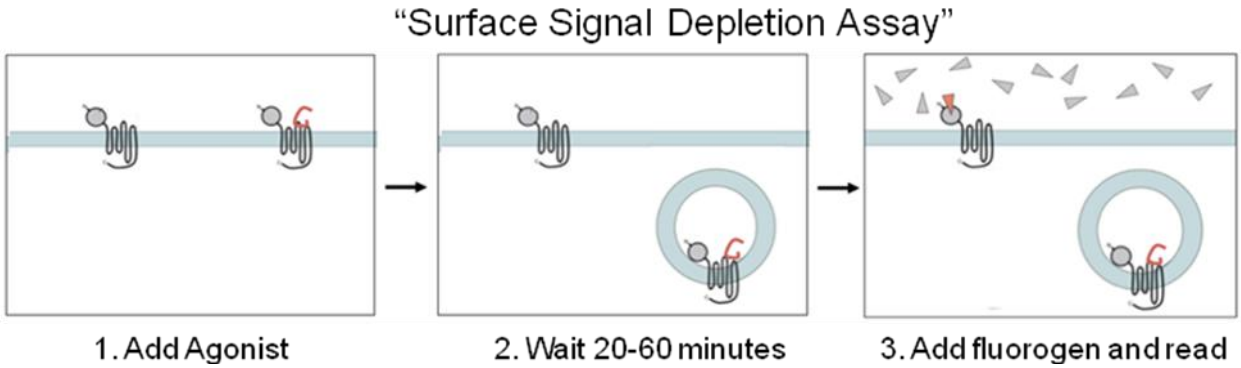
Fluorogen Activating Peptide (FAP) technology combines a genetic tag (to tag the target GPCR), and a fluorogenic dye that only gives signal when bound to the tag.

Using the FAP and a cell-impermeant dye you can selectively label cell-surface protein as shown below for ADRB2 in NIH-3T3 cells.

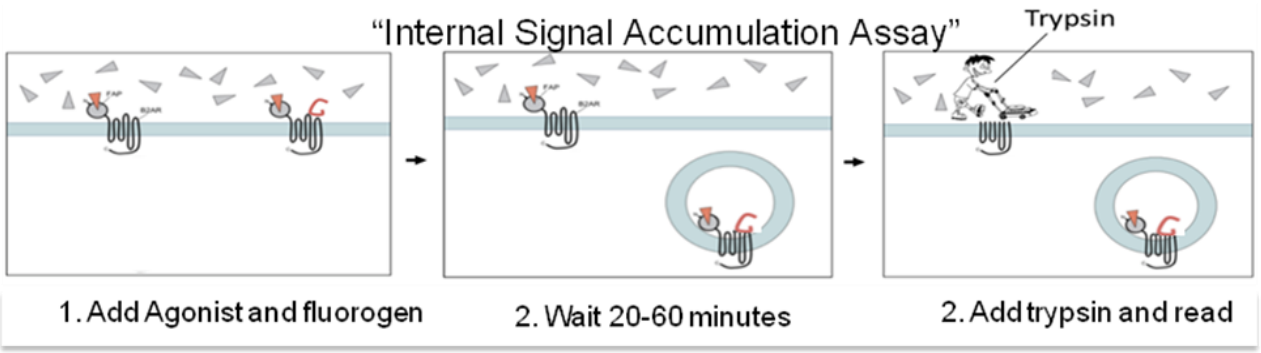
1) Using cells expressing FAP-tagged ADRB2, you first measure the surface signal from unstimulated cells by simply adding the fluorogen. This labels only the surface protein, and not protein in the Golgi/ER, or endocytic pathway, as would be the case for GFP. The confocal image at right shows this selective cell-surface labeling.	 *Surface Label at T-zero*	
2) If you add agonist to cells that have been exposed to fluorogen, the internalization process begins and the labeled receptor is now distributed between the surface, and the endosomes, as can be seen in the figure at right. In this case a significant amount of the receptor has internalized.	 *Internalization*	

Legend: Red Fluorogen (emits only when bound to FAP), Unbound extracellular Red Fluorogen (non-fluorescent when free in solution), Agonist.

The technology can be used to measure receptor internalization by either measuring the depletion of receptor at the cell surface or by measuring the accumulation of the receptor inside the cell.

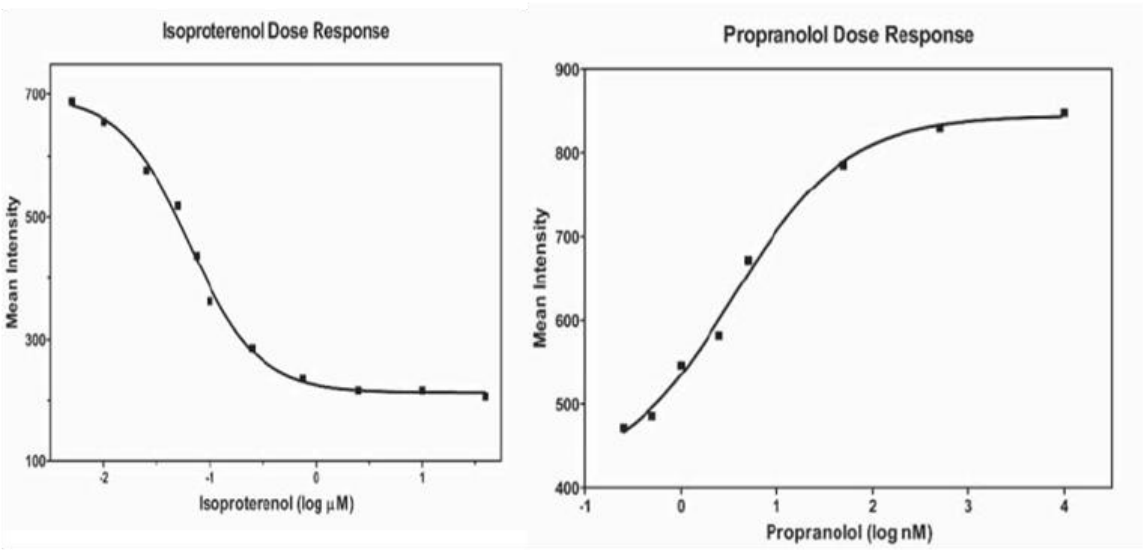


The change in signal at the surface (decrease) is proportional to the amount of receptor that internalized due to presence of agonist.



The second assay removes the surface signal leaving behind only signal from internalized protein. Only the protein that was at the surface and internalized will be fluorescent.

Both assays can be used to measure agonists or antagonist and both provide homogeneous signal, so imaging is not required. The plots (data acquired on a BD FACS-Vantage Cytometer) on the following page show data for both an agonist and an antagonist for ADRB2 using the surface depletion assay.



The left-hand panel shows decreasing surface fluorescence of ADRB2 in the presence of increasing concentration of the agonist (isoproterenol). Similarly, the right-hand panel shows increasing surface fluorescence ADRB2 when an antagonist is added (propranolol).

The following products were used to obtain the data in this report (only 1 cell line would be necessary to run the assay):

Product Name	Catalog	Amount	# Wells	Price
βGREEN fluorogen	βGREEN-np-010	20 nmol	1,000	$999
αRED fluorogen	αRED-np-010	20 nmol	1,000	$999
ADRB2-FAPα1-NIH3T3 Cell Line	ADRB2-FAPα1-CL2	2 vials	n/a	$7,450
ADRB2-FAPα1-U937 Cell Line	ADRB2-FAPα1-CL1	2 vials	n/a	$7,450
ADRB2-FAPβ1-NIH3T3 Cell Line	ADRB2-FAPβ1-CL2	2 vials	n/a	$7,450
ADRB2-FAPβ1-U937 Cell Line	ADRB2-FAPβ1-CL1	2 vials	n/a	$7,450

SpectraGenetics has tagged more than 150 GPCRs and has validated more than 30 GPCR cell lines.

We also offer this assay as a service through our partner Sharp Edge Labs (www.sharpedgelabs.com).