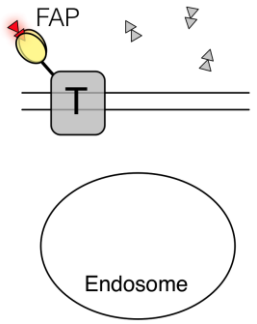
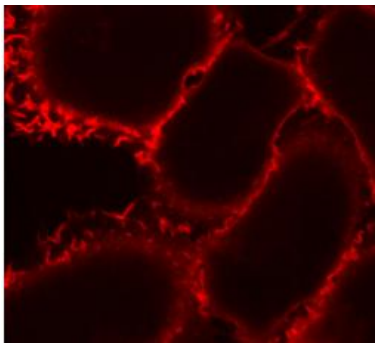
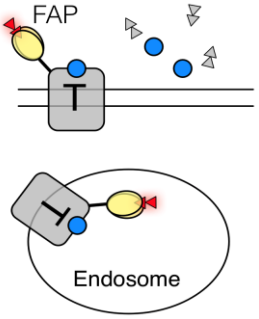
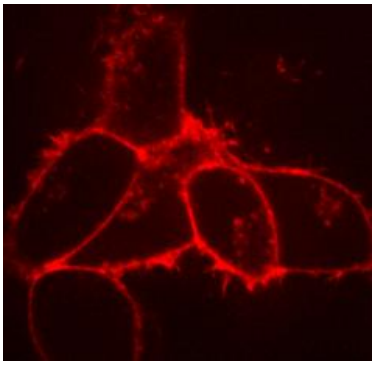
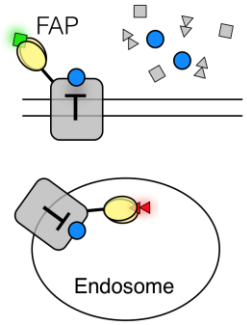
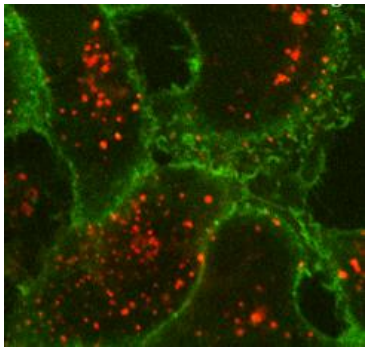
www.spectragenetics.com 412-488-9350 sales@spectragenetics.com

A Two-Color ADRB2 Internalization Assay Using FAP®-tags

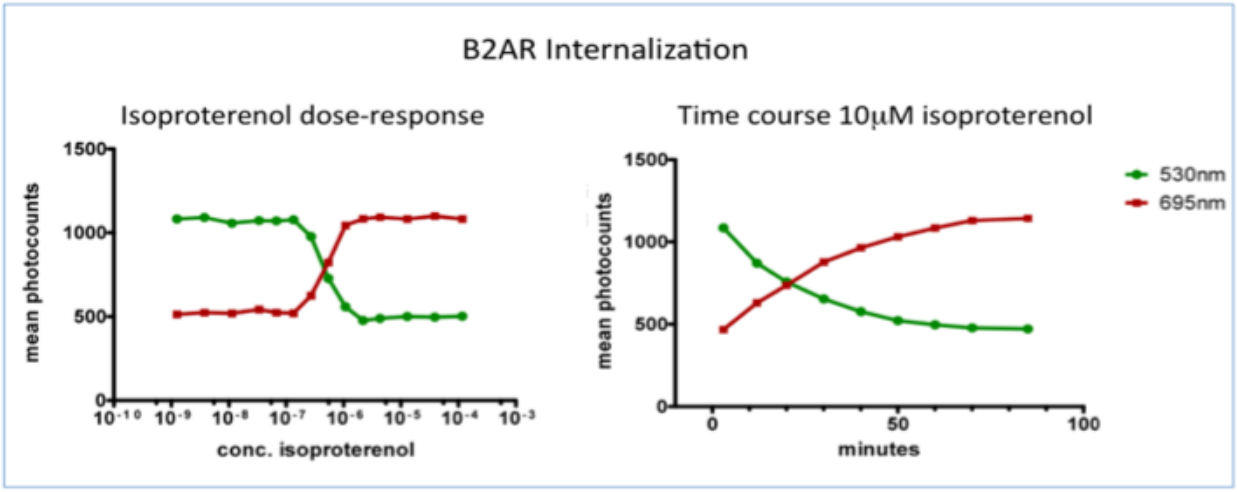
Fluorogen Activating Peptide (FAP) technology combines a genetic tag (to tag the target GPCR), and a fluorogenic dye that only gives signal when bound to the tag.

Using the FAP and cell-impermeant dyes you can selectively label cell-surface and endocytosed protein pools in the following wash-free protocol.

1) Using cells expressing FAP-tagged ADRB2, you first add a Red Fluorogen excited by the 488 laser. 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) Addition of agonist begins the internalization process, and the initial Red label is now distributed between the surface, and the endosomes, as can be seen in the figure at right. Note that agonist can be added at step one, since the Red Dye binds essentially instantaneously.	 *Internalization*	
3) At a set timepoint (40 minutes in this case), you add the Competing Fluorogen which is also excited at 488nm, but emits in the Green. This second dye is also cell impermeant, but has a higher affinity for the FAP tag, and will thus displace the Red Dye, but only for the surface protein. Red = Internal, Green = Surface	 *Fluorogen Exchange*	

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

While the confocal images above confirm that the localization of the Green and Red signal to surface and endocytic pools respectively, the signal produced in the assay is homogeneous, so imaging is not required. The plots below show the assay run on a BD FACS-Vantage cytometer, and are plots of the average fluorescence intensity from the red and green channels from 10,000 events per data point.

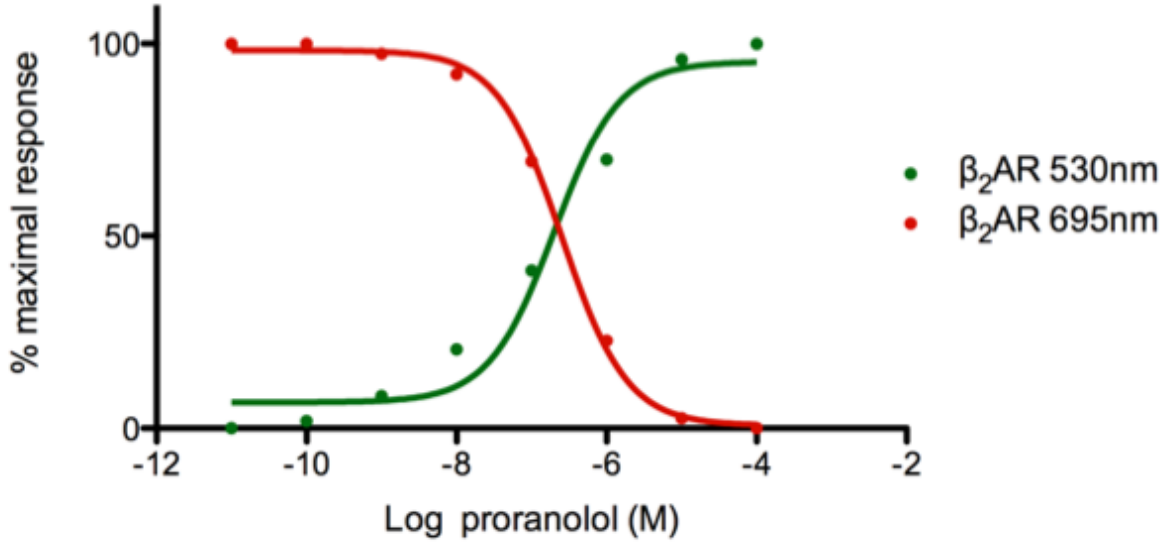


The left-hand panel shows a dose response of the receptor agonist isoproterenol. Increasing concentrations of agonist move the cells from predominantly surface GPCR localization (labeled in Green after the color-exchange), to predominantly endocytosed GPCR (the Red fluorogen, protected from exchange within the endosome).

Similarly, the right-hand panel shows a time-course following agonist addition, demonstrating a smooth transition from mostly surface GPCR at T-zero, through a clear 50/50 point at around 20 minutes, to a predominantly endocytic localization after 80 minutes.

While it is possible to simply measure an increase in the Red channel to follow agonist-driven endocytosis, measuring the Green channel simultaneously allows you to take the Red/Green ratio or Green-Red difference to obtain a larger assay window.

The same assay can be used to measure antagonist response or to see internalized receptor return to the surface (re-sensitization).



This plot shows the ratio of endocytosed and surface protein with an increasing concentration of a known antagonist (propanolol) after stimulation with isoproterenol. At higher concentration we see that the receptor recycles back to the surface.

The following products were used to obtain the data in this report:

Product Name	Catalog	Amount	# Wells	Price
βGREEN fluorogen	βGREEN-np-010	20 nmol	1,000	$999
βRED fluorogen	βRED-np-010	20 nmol	1,000	$1,499
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).