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<b>Catalog Number:</b>	RA14112	<b>Host:</b>	Rabbit
<b>Product Type:</b>	Affinity purified antiserum	<b>Species Reactivity:</b>	Rat, Mouse
<b>Immunogen Sequence:</b>	(C)TDSFSEATNV-COOH	<b>Format:</b>	1.14 mg/ml In PBS, 50% glycerol, <0.05% Sodium Azide
<b>Applications:</b>	Immunohistochemistry 1:500 -1:1000 Immunofluorescence: 1:3,000 using TSA Kit Western Blot: see DOI: 10.1002/cne.22608 Dilutions listed only as a recommendation. Optimal dilution should be determined by investigator.		
<b>Storage:</b>	Store frozen. Aliquot as undiluted serum and immediately place at -20°C. Serum may have become trapped in top of vial during shipping. Centrifugation of vial is recommended before opening. Stable for at least 6 months at -20°C. Repeated freeze/thaw cycles compromise the integrity of the antiserum.		
<b>References</b>	Andres Acosta, Maria D. Hurtado <sup>1</sup> , Oleg Gorbatyuk, Michael La Sala, David Duncan, George Aslanidi, Martha Campbell-Thompson, Lei Zhang, Herbert Herzog, Antonis Voutetakis, Bruce J. Baum, Sergei Zolotukhin. <a href="#">Salivary PYY: A Putative Bypass to Satiety. PLoS ONE 6(10): e26137. doi:10.1371/journal.pone.0026137.</a> Received: July 22, 2011; Accepted: September 20, 2011; Published: October 10, 2011		

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### Application Notes

**Immunohistochemistry:** Antiserum was used on perfusion fixed tissue. Perfusion: 1) calcium-free Tyrode's solution, 2) paraformaldehyde-picric acid fixative, and 3) 10% sucrose in PBS as a cryo-protectant. Desired tissues were dissected and stored overnight in 10% sucrose in PBS.

Slide-mounted tissue sections were processed for indirect immunofluorescence. Slides were incubated with blocking buffer for 1 hour at room temperature. Primary antiserum was diluted with blocking buffer to the appropriate working concentration. Blocking buffer was removed and slides were incubated for 18-24 hours at 4°C with primary antiserum. Slides were rinsed 3 times and then incubated with secondary antibodies for 1 hour at room temperature. Slides were again rinsed 3 times and coverslipped. Staining was examined using fluorescence microscopy.

### Immunofluorescence:

Tissues were harvested from fasted animals and immediately frozen. 4 mM thick sections were cut using a cryostat (Leica CM3050 S; Leica Microsystems, Nussloch GmbH, Germany) and then fixed in 4% paraformaldehyde for 10 min. Y2R immunolocalization was done with the TSA kit (Perkin Elmer). Tissues were blocked in 0.9% H<sub>2</sub>O<sub>2</sub> in TBS for 30 min followed by blocking with TNB (0.1 M Tris-HCl, pH 7.5, 0.15 M NaCl and 0.5% Blocking Reagent from Perkin Elmer), incubation with rabbit anti-Y2R in TBS, incubation with goat anti-rabbit MACH 2 HRP-polymer (Biocare Medical) and detection with Fluorescein provided in the TSA kit (1:300). [See doi:10.1371/journal.pone.0026137.](#)

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Images: A) Immunolocalization of Y2R-positive cells in the hippocampus of C57Bl/6J mouse (WT), a (+) control. (B) Immunolocalization of Y2R in the tongue epithelia of Y2R KO mouse, a (-) control. VEG – von Ebner's gland. (C) Immunolocalization of Y2R-positive cells in the CV area of the tongue of a C57Bl/6J mouse. (D) close-up of (C). (E), and (F) close ups of (D), top and bottom rectangles, respectively.  
doi:10.1371/journal.pone.0026137.g003

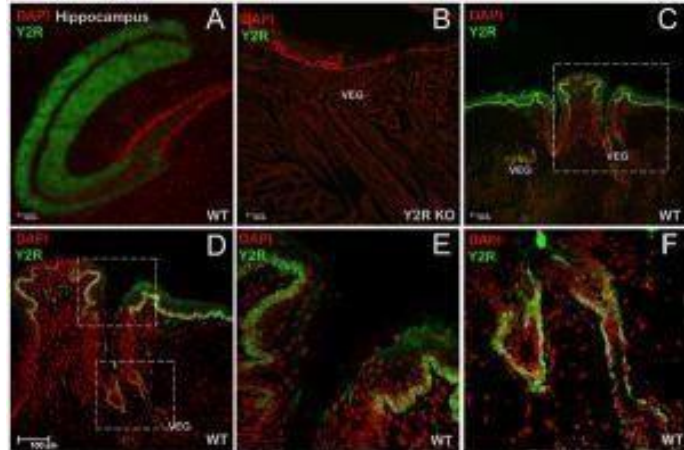
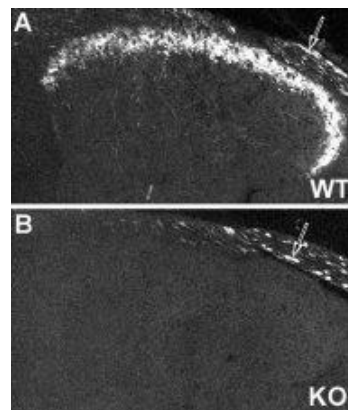


Image of NPY Y2 receptor immunoreactivity in mouse dorsal root ganglion of wild type (A) and NPY Y2 receptor Knockout (B) animals. Note that unspecific staining is observed in thick, possibly myelinated fibers. Image courtesy of Dr. Tomas Hökfelt, Karolinska Institute.



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