

Important Product News

Date: April 5th 2010

Product Change Notification

Dear Valued Customer,

We have increased the unit size (μg of protein per unit) of this Membrane Target Systems™ product in order to improve assay robustness and generate higher signals and windows. **It should be noted that the new product is identical to the previous product except for the increase in material provided.**

The affinities (Kd and Ki) have been re-measured in the new conditions. In some cases, this resulted in an apparent decrease in affinity due to an increased level of ligand depletion. This shift in affinity is solely due to the increased unit size.

Customers that are interested in obtaining affinity values more consistent with the previous product can decrease the unit size in assays. This decrease will however result in lower signals and assay windows.

To view our complete product offering for GPCRs please visit: www.perkinelmer.com/GPCR

Sincerely,

Sandra Yukie FLORES, Ph.D.
Associate Product Manager
Bio-discovery



**Any questions please contact Technical Support.
techsupport@perkinelmer.com**

Caution: For Laboratory Use. A research reagent for research purposes only

human Vasoactive Intestinal Peptide Receptor

Product No.: RBHVIPM400UA

Lot No.: 1812334

Material Provided

Membranes: 1 x 400 units / 400 µL frozen aliquot

Product Information

Cellular Background: HT-29

GenBank Accession Number: Endogenous

Unit Size: 10 µg protein / unit

Storage Buffer: 50 mM Tris-HCL (pH 7.4), 0.5mM EDTA, 10mM MgCl₂, 10% sucrose.

Storage Conditions: Store at -80°C. **Freeze-thaw is not recommended** as it can affect product performance and homogeneity. In order to minimize negative impact of freeze-thawing, flash freeze in liquid nitrogen for 30 seconds prior to transferring to -80°C.

Stability: This product is stable for at least 3 years from reception if used and stored under recommended conditions.

Quality Control

B_{max} and K_d are determined using radioactive saturation binding assays (Figure 1). Protein concentration is determined using the BCA method ⁽¹⁾. Ratio-to-Reference (RTR) is determined by dividing the maximal signal of the current lot (B_{max} in fmoles) by the maximal signal of a pre-defined reference tested in parallel. RTR is an indicator of lot-to-lot consistency. *We certify that these results meet our quality release criteria.

Ratio-to-Reference (RTR): N/A

Expression Level (B_{MAX}): 3.25 pmol/mg membrane protein.

K_D for [¹²⁵I]-Vasoactive Intestinal Peptide : 1.48 nM

Protein Concentration: 10 µg/µL

(1) Smith, P.K., et al. (1985). *Anal. Biochem.* **150**, 76-85.

Recommended Assay Conditions

Assay Buffer: 20 mM Hepes pH 7.7, 5 mM MgCl₂, 1.0% BSA

Wash Buffer: 20 mM Hepes pH 7.7, 5 mM MgCl₂, 0.15 M NaCl, 1% BSA

Binding Protocol: Binding assays are performed in 200 μ L total volume according to the following conditions:

1 - Membrane dilution: 0.05 mL of membranes + 7.45 mL assay buffer (1:150 dilution)

2 - Incubation: 25 μ L of incubation buffer or VIP (Tocris 1911) 1 μ M final for non specific binding (Saturation binding assay)

For competition binding assay: 25 μ L of reference compounds at decreasing concentrations (see figure 2)

25 μ L of radioligand at the appropriate concentration (see graph below)
150 μ L of diluted membranes

3 - Incubation time: 30 minutes at 27 $^{\circ}$ C

4 - Filtration: aspirate and wash 9 x 500 μ L with ice cold wash buffer over GF/C filter (presoaked in 0.5 % PEI).

Lot Specific Data

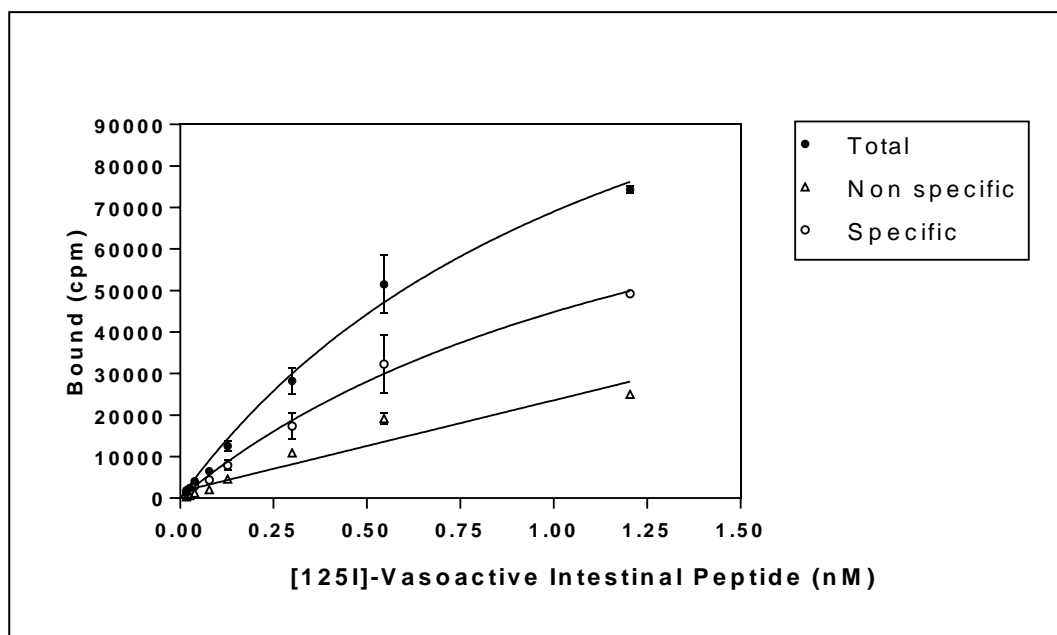


Figure 1: Saturation binding assay curve (filtration)

96-well saturation binding assay curve (10 μ g membranes/well, TopCount[®]) using [¹²⁵I]-Vasoactive Intestinal Peptide (PerkinElmer NEX192 Lot No.: CYA0130)

Typical Product Data

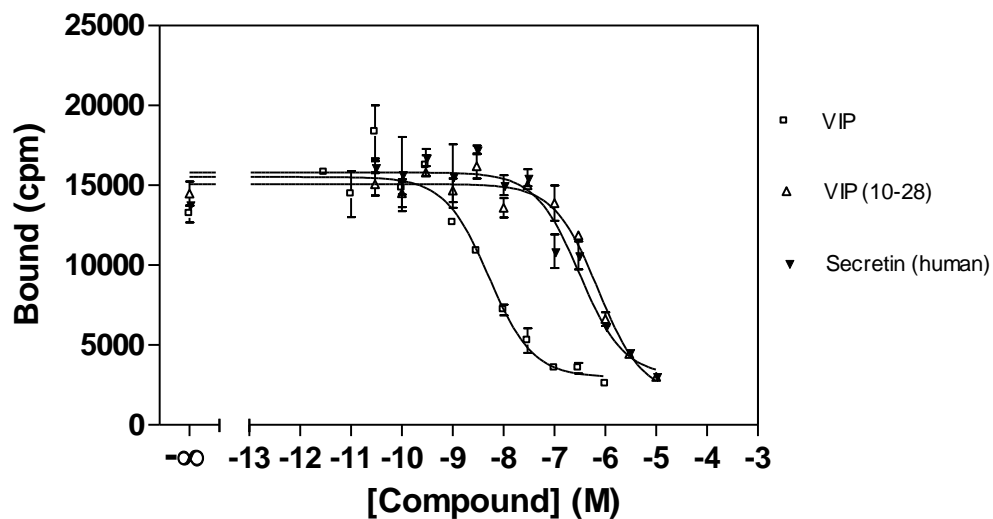


Figure 2: Competition binding assay curve (filtration)

96-well competition binding assay curve (10 μ g membranes/well, TopCount®). Recommended radioligand concentration = 0.5 nM.

*Even though two sites can be observed occasionally with some ligands, the data presented is derived from single site fitting.

Reference Compounds	K _i (nM)
VIP	4.1
VIP (10-28)	578
Secretin (human)	250

Suggested Materials and Instrumentation

Please visit our website

www.perkinelmer.com/GPCR

This product is not for resale or distribution except by authorized distributors.

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