DELFIA® neurotensin receptor binding kit AD0257

For Research Use Only

NOTE: This kit includes two packages that are sent in different temperatures. The receptor package (RBXNT1M100UA) is sent in dry ice, and the reagent package is sent at +4 °C. The reagent package should be opened immediately after receiving, and the kit components should be stored as indicated on the component labels. Part of reagents in the reagent package should be stored at -20 °C.

Because the reagents are shipped as two different shipments please check that you have both packages before you start working.

INTENDED USE

The DELFIA® neurotensin receptor binding kit is intended for setting up a neurotensin receptor binding assay.

INTRODUCTION

Human neurotensin, a 13 amino acid long peptide, exerts neuromodulatory functions in the central nervous system and endocrine/paracrine actions in the periphery. Three subtypes of neurotensin receptors have been cloned so far: NT1, NT2 and NT3. NT1 and NT2 belong to the family of G-protein coupled receptors, whereas the third one is an entirely new type of neuropeptide receptor. All three receptors bind neurotensin through its C-terminal hexapeptide sequence RRPYIL-OH.

DELFIA europium (Eu)-labeled neurotensin is a synthetic peptide, similar to human neurotensin with a europium chelate coupled to the amino end of the peptide. The sequence of DELFIA Eu-labeled neurotensin is the following: Ac-Eu^{3+}-ELTENKPRRPYIL-OH.

PRINCIPLE OF THE ASSAY

The DELFIA Eu-labeled neurotensin binding assay is based on dissociation-enhanced time-resolved fluorescence. DELFIA Eu-labeled neurotensin and the ligand specific receptor are incubated on an AcroWell[1] filter plate, after which the unbound labeled ligand is removed. Eu is dissociated from the bound ligand by using DELFIA Enhancement Solution. Dissociated Eu creates highly fluorescent complexes, which are measured in a multilabel counter with TRF option.

DELFIA is a registered trademark of PerkinElmer, Inc.
[1] AcroWell is a trademark of Pall Corporation.

KIT CONTENTS

This kit is enough for 2x96 microtiter well assays. When the receptor is used as part of DELFIA neurotensin receptor binding kit (prod. no. AD0257), 100 UA is enough for 2x96 microtiter well assays. Note that the receptor is sent separately in dry ice apart of the rest of the DELFIA kit.

Reagent package

Component	Quantity	Storage and shelf life
DELFIA Eu-labeled neurotensin 40 pmol	1 vial, lyophilized	+2 - +8 °C

The lyophilized DELFIA Eu-labeled neurotensin contains Tris-HCl buffered salt solution, bovine serum albumin (BSA), and Dextran T-40.

NOTE: The powder contains sodium azide (< 1 %) as preservative and it is harmful by inhalation, in contact with skin and if swallowed.

Unlabeled human neurotensin 3000 µmol/L	1 vial, 12 nmol	-20 °C. Avoid repeated freezing and thawing. NOTE: For maximum recovery of the product, centrifuge or shake down the original vial prior to removing the cap.

A ready-for-use solution in 50 mM Tris-HCL with 0.1 % BSA.

DELFIA L*R binding buffer concentrate (10x)	1 bottle, 5 mL	+2 - + 8 °C until expiry date stated on the bottle label.

A 10-fold concentration of Tris-HCl buffered (pH 7.5) salt solution with 2 % BSA, and < 0.1% sodium azide as preservative.

DELFIA L*R wash concentrate (25x)	1 bottle, 15 mL	+2 - + 8 °C until expiry date stated on the bottle label.

A 25-fold concentration of Tris-HCl buffered (pH 7.5) salt solution. Contains < 0.1% sodium azide as preservative.

Enhancement Solution	1 bottle, 50 mL	+2 - + 8 °C until expiry date stated on the bottle label. Shelf life 6 months at room temperature (+20 - +25 °C). Protect from light when not in regular use.

Ready-for-use solution with Triton X-100[2], acetic acid and chelators.

AcroWell Filter Plate	2 plates	+20 - +25 °C

Note: The whole plate should be used at the same time. Empty wells cannot be used afterwards because they become wet during washing.

Receptor package (RBXNT1M100UA)

Component	Quantity	Storage and shelf life
Human neurotensin NT_1 receptor 100 microassays	1 vial, enough for 192 DELFIA assays	-80 °C. Avoid repeated freezing and thawing.

Membranes suspended in 50 mM Tris-HCl (pH 7.5), and 10 % sucrose.

PREPARATION OF REAGENTS

Reagent	Storage and reconstituted stability
DELFIA Eu-labeled neurotensin	Keep the vial on ice. Stable for 5 days at +2 - +8°C. For longer periods, aliquot and store at -20°C. Avoid freezing and thawing. Stable for at least one month at -20°C.

Reconstitute the lyophilized DELFIA Eu-labeled neurotensin by adding 200 µL of distilled water to yield a DELFIA Eu-labeled neurotensin concentration of 200 nmol/L and mix gently. Allow to stand for at least 30 minutes on ice before use to ensure that all solid material is dissolved.

NOTE: The powder contains sodium azide (< 1 %) as preservative and it is harmful by inhalation, in contact with skin and if swallowed. The dissolved ligand contains < 0.1 % sodium azide and is not considered harmful.

[2] Triton is a registered trademark of Rohm and Haas Co.

L*R binding buffer	Prepare only the amount needed within one day.

Dilute DELFIA L*R binding buffer concentrate (10x) 1:10 with distilled water.

L*R wash solution	Prepare only the amount needed within one day.

Dilute DELFIA L*R wash concentrate (25x) 1:25 with distilled water.

MATERIALS REQUIRED BUT NOT SUPPLIED WITH THE KIT

The DELFIA Eu-labeled neurotensin binding assay requires the following items, which are available from PerkinElmer Life and Analytical Sciences or its distributors.

1. Time-resolved fluorometer, e.g. 2101 EnVision™, 1420 VICTOR™ or Fusion™ Multilabel Reader
2. Automatic shaker - DELFIA Plateshake (prod. no. 1296-003/004)
3. Pipette for dispensing the DELFIA Enhancement Solution - Eppendorf Multipette (prod. no. 1296-014) with 5 mL Combitips (prod. no. 1296-016) or alternatively the DELFIA Plate Dispense (prod. no. 1296-041)

In addition to the DELFIA system the following are required:

- distilled water
- filterplate washing manifold: Multiscreen Vacuum Manifold, Millipore
- precision pipettes for dispensing microliter volumes
- pipettes for dispensing milliliter volumes
- glass or polypropylene tubes

PROCEDURAL NOTES

1. When filtering the plate, ensure that each well is washed thoroughly, e.g. four times with 300 µL of wash solution. After washing the plate, check that the wells are dry. Remove any remaining moisture by blotting the plate on absorbent paper.

2. When using the Millipore manifolder/vacuum unit, the AcroWell Filter Plate may not fit tightly on the manifolder/vacuum frame. The plate will fit better on the frame if the metal grid is removed from the top of the frame. The black rubber part should, however, be left on the frame in order to prevent problems with vacuum leakage.

3. For optimal results, prior to dispensing DELFIA Enhancement Solution flush the pipette tips or the dispenser tips and tubing thoroughly with DELFIA Enhancement Solution. We recommend using plastic vials instead of glass vials.

ASSAY PROCEDURE

Two different experimental setups with DELFIA neurotensin receptor binding kit will be described below. The saturation protocol (to determine the K_d value) and the competition protocol (to determine the K_i value) are designed for research and evaluation purposes. The total assay volume is 100 µL.

Buffer composition

DELFIA L*R binding buffer

DELFIA L*R binding buffer concentrate (10x), diluted 1:10 containing the following reagents as final concentrations:

- 50 mmol/L Tris-HCl, pH 7.5
- 5 mmol/L $MgCl_2$
- 25 µmol/L EDTA
- 0.2 % BSA

DELFIA L*R wash solution

DELFIA L*R wash concentrate (25x), diluted 1:25 containing the following reagents as final concentrations:

- 50 mmol/L Tris-HCl, pH 7.5
- 5 mmol/L $MgCl_2$

Saturation protocol

1. Prepare DELFIA Eu-labeled neurotensin at 4x final concentration (4 x 4 nmol/L = 16 nmol/L) in binding buffer.

2. Dilute this 16 nmol/L DELFIA Eu-labeled neurotensin stock five times using serial doubling dilutions. Perform the serial dilutions in binding buffer (see table in the Summary Protocol Sheet).

3. Prepare the unlabeled neurotensin for non-specific binding determination at 4x final concentration (4 x 800 nmol/L = 3200 nmol/L) in binding buffer.

4. Thaw the receptor vials rapidly.

   For 1-plate assay: Dilute half of the contents of the receptor vial into 5 mL in binding buffer.

   For 2-plate assay: Dilute the contents of the receptor vial into 10 mL in binding buffer.

   Homogenize and keep on ice.

5. Add 25 µL of binding buffer to 18 wells (A1-A3, …C1-C3, A7-A9, …C7-C9, see the plate map below) for total binding determination.

6. Add 25 µL of unlabeled neurotensin to 18 empty wells (A4-A6, …C4-C6, A10-A12, …C10-C12) for non-specific binding determination.

7. Add 25 µL of DELFIA Eu-labeled neurotensin from each of the six different dilutions, using six wells per concentration (three wells for total binding and three wells for non-specific binding, A1-A12, …C1-C12).

8. Add 50 µL of diluted receptor preparation to all 36 wells.

9. Shake the plate for 15 seconds with slow shaking using the DELFIA Plateshake.

10. Incubate the plate for 90 minutes at room temperature.

11. Aspirate and wash the filter plate in a vacuum manifold with 4 x 300 µL of wash solution.

12. Add 200 µL of DELFIA Enhancement Solution directly from the reagent bottle to each well using a proper dispenser. Flush the tip for five times with DELFIA Enhancement Solution (to waste). Avoid touching the edge of the well or its contents.

13. Incubate the plate for 15 minutes at room temperature with slow shaking.

14. Measure the fluorescence with a time-resolved fluorometer.

Final concentrations of DELFIA Eu-labeled neurotensin in well (nmol/L):

	1 (+)	**2 (+)**	**3 (+)**	**4 (-)**	**5 (-)**	**6 (-)**	**7 (+)**	**8 (+)**	**9 (+)**	**10 (-)**	**11 (-)**	**12 (-)**
A	0.125	0.125	0.125	0.125	0.125	0.125	0.25	0.25	0.25	0.25	0.25	0.25
B	0.5	0.5	0.5	0.5	0.5	0.5	1.0	1.0	1.0	1.0	1.0	1.0
C	2	2	2	2	2	2	4	4	4	4	4	4

(+) = total binding (-) = non-specific binding

Competition protocol

1. Prepare DELFIA Eu-labeled neurotensin at 4x final concentration (4 x 1.0 nmol/L = 4.0 nmol/L) in binding buffer. (K_d is recommended as the final concentration.)

2. Prepare the unlabeled neurotensin at 4x final concentration (4 x 800 nmol/L = 3200 nmol/L) in binding buffer.

3. Dilute the 3200 nmol/L unlabeled neurotensin stock 10 times using serial fourfold dilutions. Perform the serial dilution in binding buffer (see table in the Summary Protocol Sheet).

4. Thaw the receptor vials rapidly.

   For 1-plate assay: Dilute half of the contents of the receptor vial into 5 mL in binding buffer.

   For 2-plate assay: Dilute the contents of the receptor vial into 10 mL in binding buffer.

   Homogenize and keep on ice.

5. Add 25 µL of binding buffer to three wells (A1-A3, see the plate map below) for total binding determination.

6. Add 25 µL of unlabeled neurotensin from each 11 different dilutions, using three wells per concentration (A4-A12, B1-B12, C1-C12).

7. Add 25 µL of DELFIA Eu-labeled neurotensin to all 36 wells.

8. Add 50 µL of diluted receptor preparation to all 36 wells.

9. Shake the plate for 15 seconds with slow shaking using the DELFIA Plateshake.

10. Incubate the plate for 90 minutes at room temperature.

11. Aspirate and wash the filter plates in a vacuum manifold with 4 x 300 µL of wash solution.

12. Add 200 µL of DELFIA Enhancement Solution directly from the reagent bottle to each well using a proper dispenser. Flush the tip for five times with DELFIA Enhancement Solution (to waste). Avoid touching the edge of the well or its contents.

13. Incubate the plate for 15 minutes at room temperature with slow shaking.

14. Measure the fluorescence with a time-resolved fluorometer.

Final concentration of unlabeled neurotensin in well (nmol/L):

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
A	0	0	0	7.5×10^{-4}	7.5×10^{-4}	7.5×10^{-4}	3.0×10^{-3}	3.0×10^{-3}	3.0×10^{-3}	1.2×10^{-2}	1.2×10^{-2}	1.2×10^{-2}
B	4.9×10^{-2}	4.9×10^{-2}	4.9×10^{-2}	1.95×10^{-1}	1.95×10^{-1}	1.95×10^{-1}	7.8×10^{-1}	7.8×10^{-1}	7.8×10^{-1}	3.125	3.125	3.125
C	12.5	12.5	12.5	50	50	50	200	200	200	800	800	800

CALCULATION OF RESULTS

$$S/B = \frac{\text{Total values}}{\text{Non-specific values}}$$

$$Z' = 1 - \frac{3 \times SD_{total} + 3 \times SD_{non\text{-}specific}}{\text{Mean signal}_{total} - \text{Mean signal}_{non\text{-}specific}}$$

SD = standard deviation

K_d and K_i values are calculated using GraphPad Prism® [3] software.

RESULTS

Saturation curve

Figure 1 shows typical data for measuring the saturation binding. The saturation experiment was performed with increasing amounts of DELFIA Eu-labeled neurotensin in the presence of 2.8 µg of hNT1 receptor (B_{max} 0.88 pmol/mg protein) per well. Non-specific binding was determined in the presence of 800 nmol/L unlabeled human neurotensin. A typical K_d value for DELFIA Eu-labeled neurotensin is around 1 nmol/L.

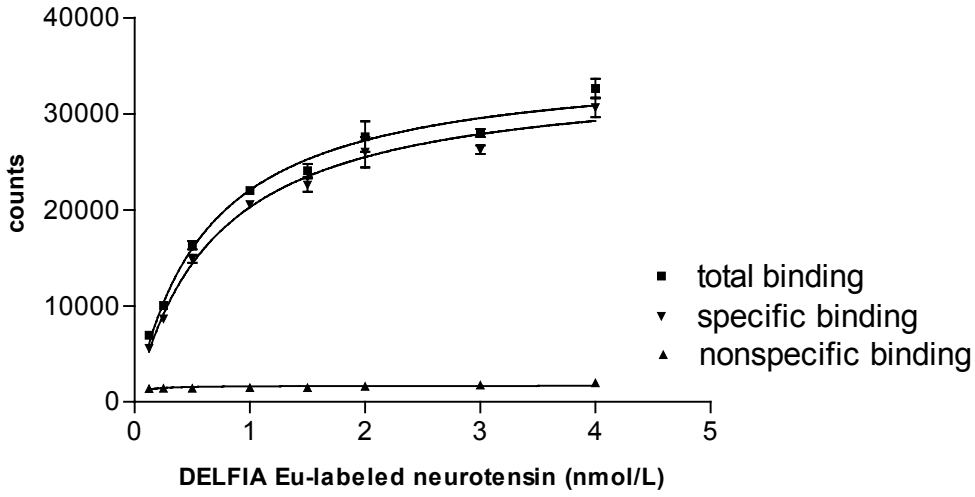

Figure 1. A K_d value of 0.70 nmol/L was obtained using the hNT1 receptor. The assay was performed as described in the section "ASSAY PROCEDURE". The fluorescence was measured with VICTOR[2]. The values represent the mean ±SD from triplicate wells.

[3] GraphPad Prism is a registered trademark of GraphPad Software Inc.

Competition curve

The competition between the DELFIA Eu-labeled neurotensin and unlabeled human neurotensin is shown in Figure 2. The displacement curve was performed with 1 nmol/L of DELFIA Eu-labeled neurotensin and increasing amounts of unlabeled neurotensin in the presence of 2.8 µg hNT1 receptor (B_{max} 0.88 pmol/mg protein) per well. A typical K_i value is around 0.5 nmol/L.

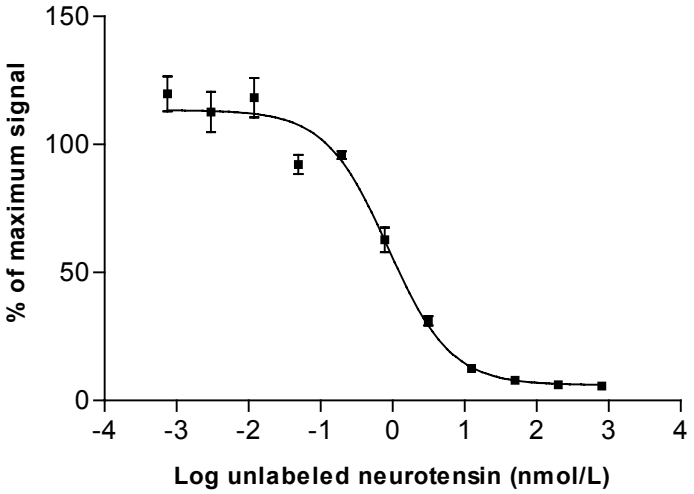

Figure 2. A K_i value of 0.43 nmol/L was obtained using the hNT1 receptor. The assay was performed as described in the section "ASSAY PROCEDURE". The fluorescence was measured with VICTOR^2. The values represent the mean ±SD from triplicate wells.

S/B ratio and Z' value

Typical readings, S/B ratios and Z' values using VICTOR and EnVision (n = 12) are shown in Table 1. S/B and Z' were calculated using a concentration of DELFIA Eu-labeled neurotensin close to the K_d value. Decreasing the number of flashes in EnVision shortens the measurement time at the expense of counts and Z' value.

Table 1. S/B and Z' were calculated using a concentration of 1 nmol/L DELFIA Eu-labeled neurotensin.

	VICTOR	EnVision	EnVision	EnVision
Flash		100	50	10
Time / plate	2 min	1 min 7 sec	55 sec	45 sec
Total binding	22986	16658	8229	1573
CV%	6.3	9.3	9.3	10.9
Non-specific binding	1280	749	369	70
CV%	7.4	7.1	9.4	10.1
S/B	18	22	22	22
Z'	0.79	0.70	0.70	0.64

WARNINGS AND PRECAUTIONS

DELFIA Eu-labeled neurotensin is intended for research use only.

Lyophilized DELFIA Eu-labeled neurotensin contains sodium azide (NaN_3) as a preservative. The powder is harmful by inhalation, in contact with skin and if swallowed. Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. On disposal, flush with a large volume of water to prevent azide build-up.

To avoid Eu-contamination that can result in a high fluorescence background in assays, high standard pipetting and washing techniques are required. Avoid contaminating pipettes with Eu-labeled reagents.

Disposal of all waste should be in accordance with local regulations.

WARRANTY

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LITERATURE

Mazor, O., Hillairet de Boisferon, M., Lombet, A., Gruaz-Guyon, A., Gayer, B., Skrydelsky, D., Kohen, F., Forgez, P., Scherz, A., Rostene, W. and Salomon, Y. (2002): Europium-labeled Epidermal Growth Factor and Neurotensin: novel probes for receptor-binding studies. Anal. Biochem. **301**, 75-81.

Zhang, J.H., Chung, T.D.Y. and Oldenburg, K.R. (1999): A simple statistical parameter for use in evaluation and validation of high throughput screening assays. J. Biomol Screen **4,** 67-73.

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To download pdf file: www.perkinelmer.com/delfia

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FINLAND

PerkinElmer®
precisely.

To order bulk amount please use the following product numbers:

Product number	Product	Package
AD0208	DELFIA Eu-labeled motilin	60 pmol* (enough for appr. 960 wells)
AD0209	DELFIA Eu-labeled motilin	240 pmol* (enough for appr. 4800 wells)
AD0213	DELFIA Eu-labeled interleukin-8	160 pmol* (enough for appr. 960 wells)
AD0214	DELFIA Eu-labeled interleukin-8	700 pmol* (enough for appr. 4800 wells)
AD0215	DELFIA Eu-labeled galanin	200 pmol* (enough for appr. 960 wells)
AD0216	DELFIA Eu-labeled galanin	850 pmol* (enough for appr. 4800 wells)
AD0217	DELFIA Eu-labeled EGF	350 pmol* (enough for appr. 960 wells)
AD0218	DELFIA Eu-labeled EGF	1400 pmol* (enough for appr. 4800 wells)
AD0219	DELFIA Eu-labeled neurotensin	200 pmol* (enough for appr. 960 wells)
AD0220	DELFIA Eu-labeled neurotensin	750 pmol* (enough for appr. 4800 wells)
AD0221	DELFIA Eu-labeled neurokinin A	300 pmol* (enough for appr. 960 wells)
AD0222	DELFIA Eu-labeled neurokinin A	1200 pmol* (enough for appr. 4800 wells)
AD0223	DELFIA Eu-labeled substance P	200 pmol* (enough for appr. 960 wells)
AD0224	DELFIA Eu-labeled substance P	800 pmol* (enough for appr. 4800 wells)
AD0225	DELFIA Eu-labeled NDP-αMSH	200 pmol* (enough for appr. 960 wells)
AD0226	DELFIA Eu-labeled NDP-αMSH	800 pmol* (enough for appr. 4800 wells)
AD0227	DELFIA Eu-labeled bombesin	150 pmol* (enough for appr. 960 wells)
AD0228	DELFIA Eu-labeled bombesin	600 pmol* (enough for appr. 4800 wells)
CR400-600	DELFIA Eu-labeled TNFα	600 pmol
CR401-650	DELFIA Eu-labeled interleukin-2	650 pmol
CR402-400	DELFIA Eu-labeled interleukin-5	400 pmol
CR403-060	DELFIA Eu-labeled interleukin-4	60 pmol
1244-104	DELFIA Enhancement Solution	50 mL
1244-105	DELFIA Enhancement Solution	250 mL
4001-0010	DELFIA Enhancement Solution	1000 mL
CR134-250	DELFIA L*R binding buffer concentrate (10x)	250 mL
CR135-250	DELFIA L*R wash concentrate (25x)	250 mL
AAAND-0005	DELFIA Streptavidin-coated yellow plate, 96 well	10 plates
RBHMOTM	Human motilin receptor	400 UA
RBHCX2M	Human recombinant interleukin-8b CXCR2 receptor	400 UA
RBHEGFM	Human endogenous epidermal growth factor receptor	400 UA
RBXNT1M	Human recombinant neurotensin receptor subtype 1	400 UA
RBXMC3M	Human recombinant melanocortin receptor MC3	400 UA
RBHMC4M	Human recombinant melanocortin receptor MC4	400 UA
RBXMC5M	Human recombinant melanocortin receptor MC5	400 UA
RBHBS1M	Human recombinant bombesin receptor subtype 1	400 UA
RBHBS2M	Human recombinant bombesin receptor subtype 2	400 UA
6110551 (Amersham)	Human endogenous neurokinin receptor subtype 1	200 UA
6110510 (Amersham)	Human recombinant neurokinin receptor subtype 2	200 UA
P5020	AcroWell Filter Plate, 96 well	10 plates
RBXGL1M	Human recombinant galanin subtype 1 GAL1	400 UA
RBXGL2M	Human recombinant galanin subtype 2 GAL2	400 UA

*The number of the wells varies depending on the assay conditions.

For customized labelling ligands please email labellingservices@perkinelmer.com.

DELFIA® neurotensin receptor binding kit
Summary Protocol Sheet

1. Preparation of reagents

Reconstitute Eu-labeled neurotensin		40 pmol	200 µL distilled water → 200 nmol/L	30 min. For longer storage at -20°C

Dilute the reconstituted Eu-labeled neurotensin in binding buffer — Keep on ice.

For saturation protocol

Conc. nmol/L	Final conc. nmol/L	Eu-labeled neurotensin	Binding buffer
16	4	40 µL of 200 nmol/L	460 µL
8	2	250 µL of 16 nmol/L	250 µL
4	1	250 µL of 8 nmol/L	250 µL
2	0.5	250 µL of 4 nmol/L	250 µL
1	0.25	250 µL of 2 nmol/L	250 µL
0.5	0.125	250 µL of 1 nmol/L	250 µL

For competition protocol

Conc. nmol/L	Final conc. nmol/L	Eu-labeled neurotensin	Binding buffer
4	1	30 µL of 200 nmol/L	1470 µL

Dilute neurotensin receptor — Keep on ice.

For saturation and competition protocols

Assays	Final conc. µassays/well	Neurotensin receptor	Binding buffer
96	0.5	Half volume of the vial	To 5 mL
192	0.5	The whole volume of the vial	To 10 mL

Continued →

Dilute the unlabeled neurotensin in binding buffer

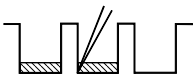
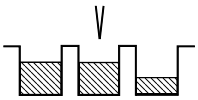
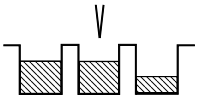
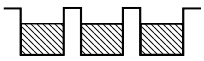
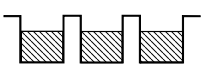
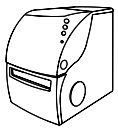
For saturation and competition protocols

Conc. nmol/L	Final conc. nmol/L	Unlabeled neurotensin	Binding buffer
3200	800	1.5 µL of 3000 µmol/L	1400 µL

For competition protocol

Conc. nmol/L	Final conc. nmol/L	Unlabeled neurotensin	Binding buffer
800	200	75 µL of 3200 nmol/L	225 µL
200	50	75 µL of 800 nmol/L	225 µL
50	12.5	75 µL of 200 nmol/L	225 µL
12.5	3.125	75 µL of 50 nmol/L	225 µL
3.125	0.78	75 µL of 12.5 nmol/L	225 µL
0.78	0.195	75 µL of 3.125 nmol/L	225 µL
0.195	0.049	75 µL of 0.78 nmol/L	225 µL
0.049	0.012	75 µL of 0.195 nmol/L	225 µL
0.012	0.003	75 µL of 0.049 nmol/L	225 µL
0.003	0.00075	75 µL of 0.012 nmol/L	225 µL

Keep on ice.

2. Assay protocol		**Manual**
Add binding buffer or unlabeled neurotensin (or compounds)		25 µL
Add Eu-labeled neurotensin (1.0 nmol/L final conc.)		25 µL
Add diluted receptor		50 µL
Incubate		15 sec. slow shaking + 90 min. at RT
Wash in vacuum manifold		4 x 300 µL
Add Enhancement Solution		200 µL
Incubate		15 min. slow shaking
Measure TR-fluorescence		Eu-filter