# Europium-labeled Ligands for Receptor Binding Studies

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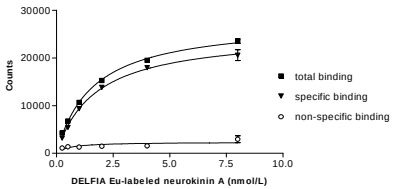
## 1 Introduction

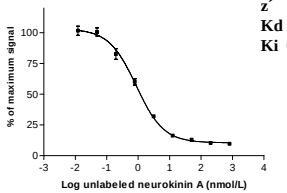
**Time-resolved fluorescence enhancement technique DELFIA® enables development of highly sensitive assays for screening. We have developed a family of Europium-labeled peptides and proteins designed for ligand receptor binding assays that can be easily automated and optimized either for 96 or 384 well format. These Eu-labeled ligands provide an excellent non-radioactive alternative that is both stabile and sensitive.**
**The new tachycinin members of the DELFIA® ligand family are Substance P and Neurokinin A. These peptide ligands have many physiological roles, e.g. they stimulate smooth muscle contraction and glandular secretion and are involved in immune responses and neurotransmission.**

## 2 Methods

**The DELFIA® ligand receptor binding assay is based on dissociation-enhanced time-resolved fluorescence. DELFIA® Eu-labeled ligand and receptor membrane preparate are incubated on an filter plate (PALL AcroWell or AcroPrep) after which unbound labeled ligand is removed by filtration. 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, e.g. EnVision™.**

## 3 DELFIA® Neurokinin A assays on 96 well filtration plates



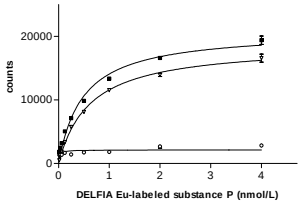

**S/B 12**
**z´ 0,73**
**Kd 1,7 nM**
**Ki 0,4 nM**

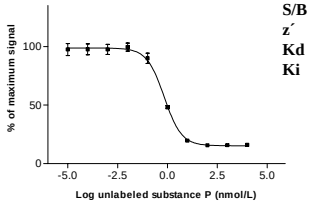
The saturation experiment was performed on a 96 well filtration plate with increasing amounts of DELFIA Eu-labeled neurokinin A in the presence of 1 µg of human NK-2 receptor ($B_{max}$ 2.8 pmol/mg protein) per well. Non-specific binding was determined in the presence of 250 nmol/L unlabeled human neurokinin A. A $K_d$ value of 1.7 nmol/L was obtained.

The competition between DELFIA Eu-labeled neurokinin A and unlabeled human neurokinin A. The displacement curve was performed with 1.7 nmol/L of DELFIA Eu-labeled neurokinin A and increasing amounts of unlabeled neurokinin A in the presence of 1 µg of human NK-2 receptor ($B_{max}$ 2.8 pmol/mg protein) per well. A $K_i$ value of 0.4 nmol/L was obtained.

**Results are comparable to radioligand binding assays**

## 4 DELFIA® Substance P assays on 96 well filtration plates




**S/B 9**
**z´ 0,67**
**Kd 0,61 nM**
**Ki 0,34 nM**

The saturation experiment was performed on a 96 well filtration plate with increasing amounts of DELFIA Eu-labeled substance P in the presence of 12 µg of **human endogenous NK-1 receptor** ($B_{max}$ 0.25 pmol/mg protein) per well. Non-specific binding was determined in the presence of 500 nmol/L unlabeled human substance P. A $K_d$ value of 0.61 nmol/L was obtained.

The competition between the DELFIA Eu-labeled substance P and unlabeled human substance P is shown in Figure 2. The displacement curve was performed with 0.7 nmol/L of DELFIA Eu-labeled substance P and increasing amounts of unlabeled substance P in the presence of 12µg of **human endogenous NK-1 receptor** ($B_{max}$ 0.25pmol/mg protein) per well. A $K_i$ value of 0.34 nmol/L was obtained.

**Results are comparable to radioligand binding assays**

## 5 Assays on 384 well filtration plates

- 5 µl unlabeled ligand
- 5 µl Eu-ligand
- 10 µl receptor
- incubation 90min (15 s shake)
- filtration wash 3x300 µl (DELFIA® L*R wash solution)
- 20 µl Enhancement Solution
- incubation 15 min (shaking)
- TRF measurement

**Kd and Ki values on 384 AcroPrep plates**

| | Kd | Ki | S/B |
|---|---|---|---|
| Neurokinin A | 0,94 | 0,45 | 9 |
| Substance P | 0,43 | 0,31 | 9 |

**Results are comparable to results on 96 well plates**

## 6 Comparision of manual and automated assay formats

**Manual protocol**
- 25 µl unlabeled ligand
- 25 µl Eu-ligand
- 50 µl receptor
- incubation 90min (15 s shake)
- filtration wash 3x300 µl
- 200 µl Enhancement Solution
- incubation 15 min (shaking)
- TRF measurement

**Automation protocol**
- 2 µl unlabeled ligand in DMSO
- 50 µl Eu-ligand
- 50 µl receptor
- incubation 2 h - 5 h
- filtration wash 3x300 µl
- 200 µl Enhancement Solution
- incubation 90 min - 4 h
- TRF measurement

| | automated assay | | manual assay | |
|---|---|---|---|---|
| | S/B | z´ | S/B | z´ |
| Neurokinin A | 6,3 | 0,73 | 7,2 | 0,69 |
| Substance P | 6,9 | 0,76 | 6,7 | 0,64 |

**Assays can be easily automated - increased through-put with less variation**

## 7 DELFIA® Eu-ligand products available

| | |
|---|---|
| **Motilin** | **IL-8** |
| **Galanin** | **IL-2** |
| **EGF** | **IL-5** |
| **Neurotensin** | **TNFα** |
| **Neurokinin A** | **Bombesin** |
| **Substance P** | **NDP-αMSH** |

**L*R binding buffer concentrate (10 x)**
**L*R wash concentrate (25 x)**

## 8 Conclusions

**The DELFIA® ligand receptor binding assay characteristics:**

**High sensitivity - works with low expression level receptors, endogenous receptors**
**Non-radioactive - no radioactive waste, long shelf life**
**Several assay formats - manual and automated, 96- and 384-well format**