# DELFIA, LANCE and TruPoint technologies

## Sensitivity and dynamic range using PerkinElmer instrumentation and microplates

### Introduction

DELFIA®, LANCE™ and TruPoint™ are all assay technologies based on time-resolved fluorometry (TRF). In this technical note the performance of several PerkinElmer microplate readers and microplates are compared using these assay technologies.

Separation-based DELFIA assays are extremely sensitive and can be multiplexed using four different lanthanides (europium [Eu], samarium [Sm], terbium [Tb] and dysprosium [Dy]). The DELFIA method is based on use of lanthanide chelate-labeled tracer molecules. The unbound fraction is washed away and the bound fraction is measured after addition of Enhancement Solution. Large macromolecules such as DNA or proteins as well as smaller molecules such as haptens and peptides can be labeled with DELFIA chelates. DELFIA assays are excellent alternatives to ELISA assays, usually resulting in better assay performance.

LANCE assays are homogeneous and based on fluorescence resonance energy transfer (FRET) between chelate donor and acceptor fluorophore. In LANCE technology the energy transfer signal increase corresponds to the number of donor and acceptor pairs in close proximity. Thus LANCE technology is applicable to assay applications where an increase in binding is followed, such as protein-protein interactions and kinase assays.

TruPoint technology relies on quenching of the lanthanide fluorescence by an organic acceptor dye. The separation of the quencher from the chelate results in a signal increase enabling sensitive homogeneous assays. TruPoint assays are developed especially for measuring separation assays, catalyzed, for example, by proteases and helicases.

This application note provides recommendations for optimal read outs of time-resolved fluorescence assays for several measurement devices and microplates. The PerkinElmer instruments tested were: VICTOR²™V, EnVision™, ViewLux™ and Fusion™. The ViewLux response is shown as ADUs (Analog to Digital Units), whereas the others are counts.

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PerkinElmer® precisely.

## Methods

### Instrument performance comparison: DELFIA

Instrument performance was tested by measuring a dilution series of Eu-labeled streptavidin in DELFIA Enhancement Solution (½-log steps from 6.3 x $10^{-4}$ g/L to 2 x $10^{-12}$ g/L). Microplates tested were white and black OptiPlate™-96, white OptiPlate-384 and DELFIA yellow 96-well microplates. Europium concentration in the sample was normalized against a 1 nM europium standard solution for accurate sensitivity determination. Microplates were measured with VICTOR²V, EnVision, ViewLux and Fusion instruments (settings presented in Table 1). For best results, any material that could contain europium, such as glass, was avoided. Pipette tips were soaked with DELFIA Enhancement Solution for 5 minutes and then filled and emptied a couple of times with the DELFIA Enhancement Solution before use.

### Instrument performance comparison: LANCE and TruPoint

The PerkinElmer LANCE Tyrosine Kinase Start-up Reagent Package (Eu-labeled anti-phosphotyrosine antibody, APC labeled Streptavidin and biotinylated positive control phosphopeptide) was used in testing. The instruments were compared with two different assays using black and white 384-well OptiPlates: 1) a dilution series of positive control and 2) diluting all the reaction components, starting from 10 nM phosphopeptide, 50 nM SA-APC and 1 nM Eu-labeled anti-P-Tyr antibody and making a series of 1:1 dilutions. Data is useful when determining the optimal assay conditions and the minimum requirements for a reliable assay. The detection sensitivities were determined for the same instruments that were tested with the DELFIA assay. Table 2 summarizes the instrument settings.

The PerkinElmer Caspase-3 Kit was used as a model for the TruPoint time-resolved fluorescence quenching assay. The kit consists of a quenched caspase-3 substrate, caspase-3 assay buffer and microplates needed for the measurement. The caspase-3 enzyme was purchased from R&D Systems (Minneapolis, MN, USA). Black and white OptiPlate-96, OptiPlate-384 and OptiPlate-1536 were tested with 200 nM substrate and different enzyme concentrations. All other assay conditions were as recommended in the kit manual. Total assay volumes used were 50, 25 and 5 mL for 96-, 384- and 1536-well

*Table 1.* Instrument settings, DELFIA

| Protocol Table | VICTOR²V | EnVision | ViewLux | Fusion |
|---|---|---|---|---|
| Measurement Height | 8.00 mm | 3.00 mm | 3.00 mm | N/A |
| Measurement Delay | 400 µs | 400 µs | 401 µs | 400 µs |
| Measurement Window | 400 µs | 400 µs | 354 µs | 400 µs |
| Measurement Cycle | 1,000 µs | 2,000 µs | 1,000 µs | N/A |
| Measurement Time or Flashes | 1 sec/well | 100 flashes/well | 30 sec/plate | 80 flashes/well |

*Table 2.* Instrument settings, LANCE and TruPoint

| Protocol Table | VICTOR²V | EnVision | ViewLux | Fusion |
|---|---|---|---|---|
| Measurement Height | 8.00 mm | 5.00 mm | 3.00 mm | N/A |
| Measurement Delay | 50 µs | 60 µs | 50 µs | 50 µs |
| Measurement Window | 100 µs | 100 µs | 354 µs | 100 µs |
| Measurement Cycle | 1,000 µs | 2,000 µs | 1,000 µs | N/A |
| Measurement Time or Flashes | 1 sec/well | 100 flashes/well | 15 sec/plate | 80 flashes/well |

*Table 3.* The europium detection sensitivities measured with DELFIA technology. Measurement time of a single plate is also presented for each instrument.

| Sensitivity Table | Device | Europium Detection Limit (M) | amol/well | Time/plate (min) |
|---|---|---|---|---|
| **White 96-well** OptiPlate | VICTOR²V | 6.3E-15 | 1.3 | 2:08 |
| | EnVision | 2.8E-14 | 5.5 | 1:06 |
| | Fusion | 1.6E-14 | 3.3 | 2:48 |
| **Black 96-well** OptiPlate | VICTOR²V | 2.7E-14 | 5.4 | |
| | EnVision | 2.1E-14 | 4.3 | |
| | Fusion | 5.6E-13 | 113.0 | |
| **Yellow 96-well** DELFIA plate | VICTOR²V | 2.2E-14 | 4.3 | |
| | EnVision | 3.0E-14 | 6.0 | |
| | Fusion | 1.1E-13 | 21.0 | |
| **White 384-well** OptiPlate | VICTOR²V | 4.9E-14 | 2.5 | 7:38 |
| | EnVision | 2.4E-14 | 1.2 | 2:41 |
| | Fusion | 6.3E-14 | 3.1 | 8:26 |
| | ViewLux | 2.6E-13 | 12.9 | 1:12 |

microplates, respectively. The instrument settings for TruPoint measurements were the same as those used with LANCE.

## Results

Instrument performances for detecting diluted europium with DELFIA are summarized in Table 3. The graphical data are presented in Figures 1, 2, 3 and 4.

Instrument performance with the LANCE Tyrosine Kinase Start-up reagents was determined using two approaches: detection sensitivity for the peptide dilution series and sensitivity for a diluted reaction (see Methods). Summary of these measurements is presented in Table 4, and the graphical data in Figures 5, 6, 7 and 8.

Summary of the LANCE sensitivity data is presented in Table 4. The lowest concentration of reagents producing a Z'-value > 0.5 was used as a measurement of sensitivity. Data show that detection sensitivity is dependent upon the type of microplate used. EnVision clearly outperforms the other instruments in terms of sensitivity and reagent consumption per well when a black microplate is used. With the white microplate the reagent costs is also lowest with EnVision (see Z'-factor at diluted reaction column).

Results from the caspase-3 enzyme titration measurements with TruPoint are presented in Figures 9, 10, 11, 12, 13 and 14. The Z'-values with 0.5 ng/mL enzyme calculated from four replicates and the measurement times with different instruments are presented in Table 5. The ViewLux was the fastest of the instruments tested with a microplate processing time of 70 seconds/plate regardless of the plate type.

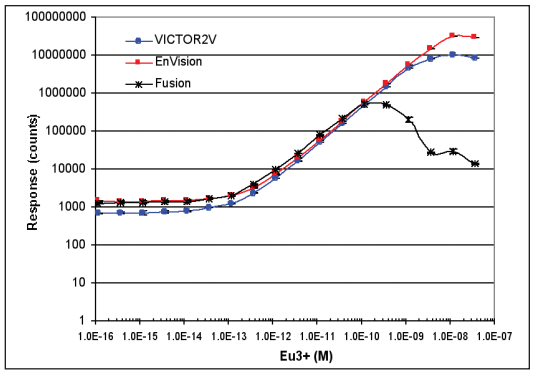


*Figure 1.*
DELFIA europium measured with white OptiPlate-96.

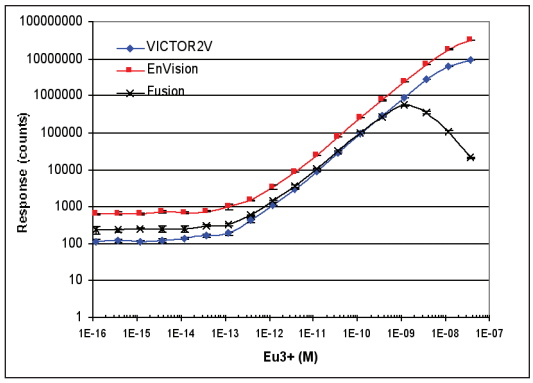


*Figure 2.*
DELFIA europium measured with black OptiPlate-96.

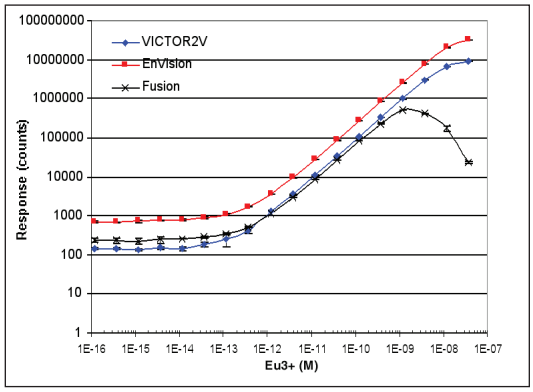


*Figure 3.*
DELFIA europium measured with yellow 96-well DELFIA plate.

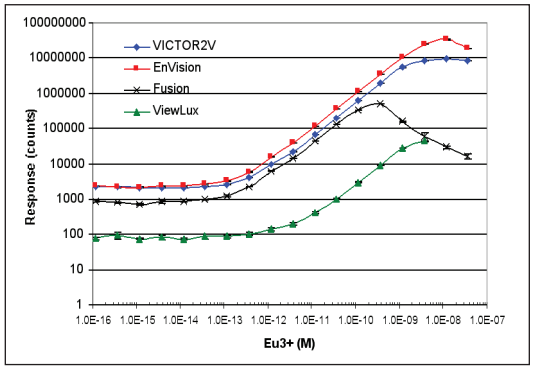


*Figure 4.*
DELFIA europium measured with white OptiPlate-384.

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Results from the caspase-3 enzyme titration measurements with TruPoint are presented in Figures 9, 10, 11, 12, 13 and 14. The Z'-values with 0.5 ng/mL enzyme calculated from four replicates and the measurement times with different instruments are presented in Table 5. The ViewLux was the fastest of the instruments tested with a microplate processing time of 70 seconds/plate regardless of the plate type.

*Table 4.* Instrument performance comparison with LANCE. Sensitivity was determined for peptide dilution series and for dilution series of the whole reaction. Assay producing a Z'-factor > 0.5 was used as a determination of minimum amount of reaction components needed. Trend is a calculation method for the detection sensitivity.

| Sensitivity Table | | Diluted Peptide (nM) | | Diluted Reaction (nM) | |
|---|---|---|---|---|---|
| | | Z' > 0.5 | Detection Limit | Z' > 0.5 | Detection Limit |
| White 384-OptiPlate | VICTOR²V | **0.111** | **0.017** | 0.155 | 0.070 |
| | ViewLux | 0.257 | 0.022 | **0.073** | **0.011** |
| | EnVision | 0.224 | 0.048 | 0.130 | 0.033 |
| | Fusion | 0.285 | 0.029 | 0.362 | 0.055 |
| Black 384-OptiPlate | VICTOR²V | 0.548 | 0.097 | 0.550 | 0.349 |
| | ViewLux | 1.193 | 0.095 | 0.638 | 0.334 |
| | EnVision | **0.242** | **0.073** | **0.295** | **0.097** |
| | Fusion | 1.068 | 0.258 | 1.833 | 0.402 |

*Table 5.* Z'-values and measurement times for different instruments and microplates in TruPoint assays

| | Z' | VICTOR²V Meas. Time* | Z' | EnVision Meas. Time | Z' | ViewLux Meas. Time | Z' | Fusion Meas. Time |
|---|---|---|---|---|---|---|---|---|
| White 96-well | 0.98 | 1.26 | 0.98 | 0.58 | 0.95 | 1.10 | 0.98 | 2.53 |
| Black 96-well | 0.90 | | 0.94 | | 0.97 | | 0.94 | |
| White 384-well | 0.90 | 3.24 | 0.93 | 2.35 | 0.89 | 1.10 | 0.98 | 8.47 |
| Black 384-well | 0.63 | | 0.69 | | 0.72 | | 0.71 | |
| White 1536-well | 0.80 | 14.35 | 0.87 | 9.16 | 0.91 | 1.10 | 0.82 | 32.02 |
| Black 1536-well | 0.75 | | 0.77 | | 0.73 | | 0.69 | |

* minutes

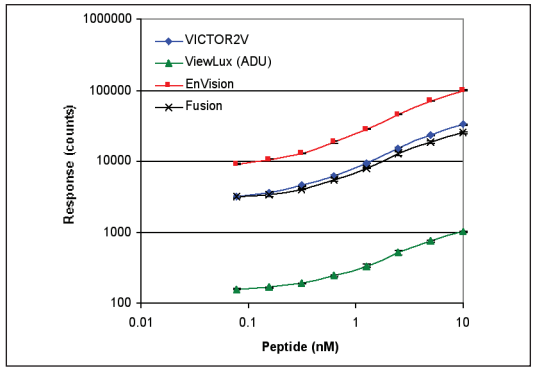


*Figure 5.* LANCE instrument performance for peptide dilution series measured with white OptiPlate-384.

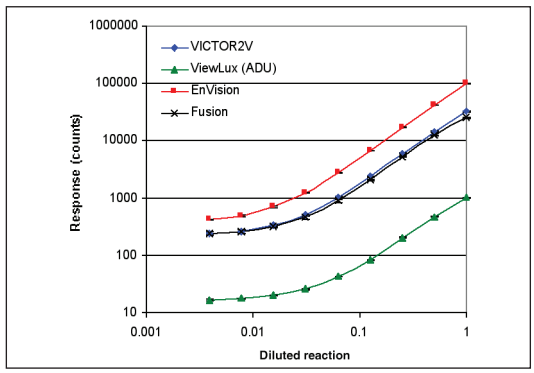


*Figure 6.* Instrument performance in LANCE for diluted reaction measured with white OptiPlate-384. X-axis presents the dilution factor where 1 is composed of 10 nM peptide, 50 nM SA-APC and 1 nM Eu-labeled anti-P-Tyr antibody diluted in the assay buffer and where 0.1 corresponds components diluted 1:10 in the reaction buffer.

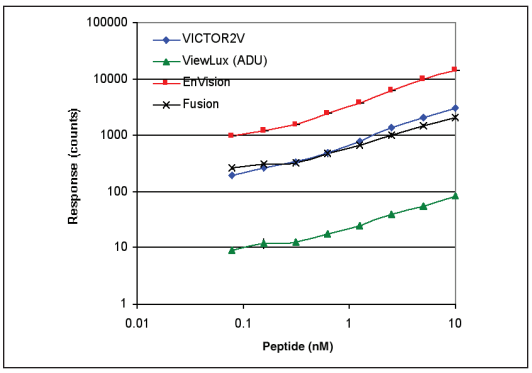


*Figure 7.* LANCE instrument performance for peptide dilution series measured with black OptiPlate-384.

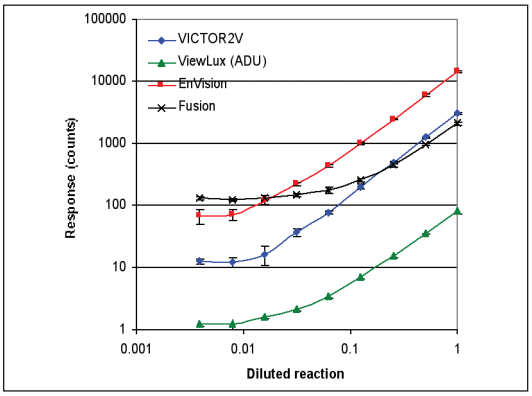


*Figure 8.* LANCE instrument performance for diluted reaction measured with black OptiPlate-384. X-axis presents the dilution factor where 1 is composed of 10 nM peptide, 50 nM SA-APC and 1 nM Eu-labled anti-P-Tyr antibody diluted in the assay buffer and where 0.1 corresponds components diluted 1:10 in the reaction buffer.

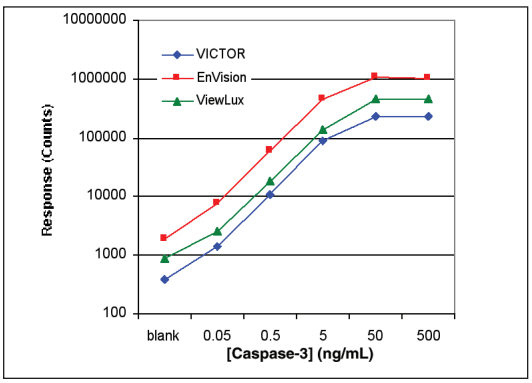


*Figure 9.* TruPoint assay with white OptiPlate-96.

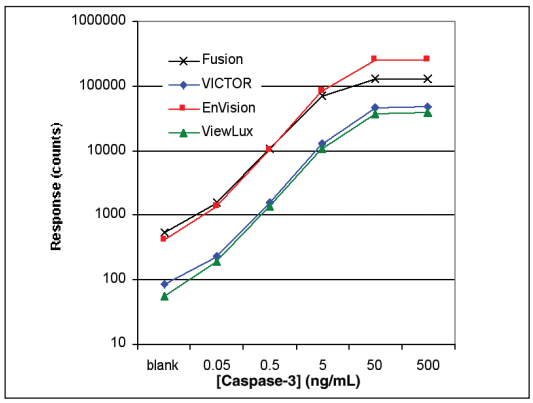


*Figure 10.* TruPoint assay with black OptiPlate-96.

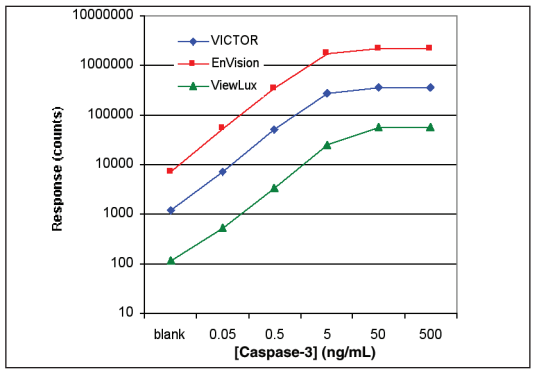


*Figure 11.* TruPoint assay with white OptiPlate-384

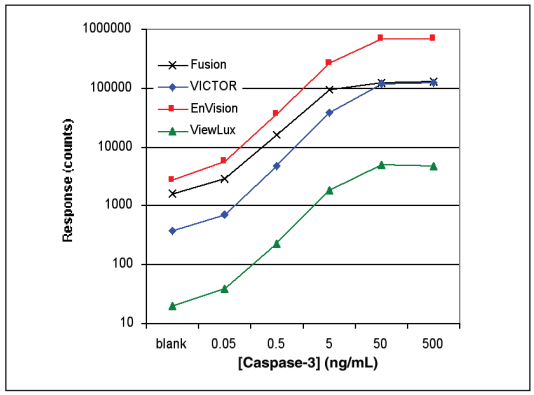


*Figure 12.* TruPoint assay with black OptiPlate-384

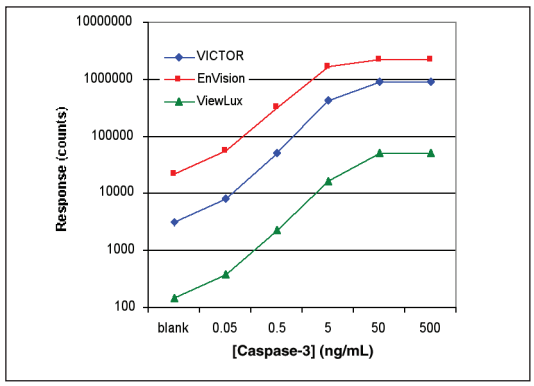


*Figure 13.* TruPoint assay with white OptiPlate-1536.

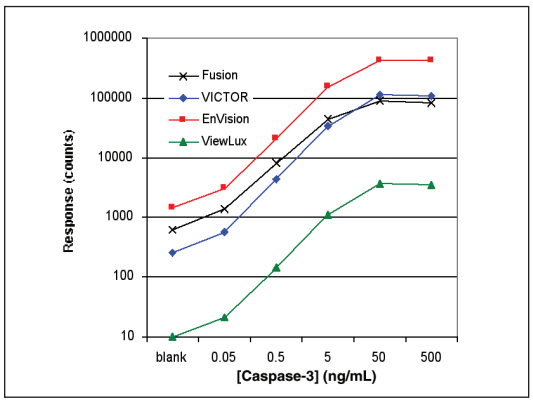


*Figure 14.* TruPoint assay with black OptiPlate-1536.

## Conclusions

DELFIA technology is a very sensitive method for quantitative analysis of proteins, DNA, RNA or even lipids using PerkinElmer multilabel counters. The detection limit for europium is clearly under 10 amol/well. There were some differences in sensitivities between the different instruments, the VICTOR and EnVision having the best sensitivity. All the instruments were compatible with all the microplates tested with DELFIA technology. Dynamic range, which is usually the most valuable factor when setting up robust assays, was about 5 decades with VICTOR and EnVision instruments, where as the Fusion was limited to about 3 decades. All the instruments showed that the best dynamic range and good detection sensitivity was obtained with the yellow microplate that is specifically designed for DELFIA technology.

LANCE technology is utilized in many applications, for example in kinase and protein-protein interaction assays as well as in hybridization assays for nucleic acids. The instrument performances for LANCE assay were evaluated by using two different methods. In the first approach the minimum target amount for detection of positive signal was determined by making dilutions of the phosphorylated peptide while the concentration of all the other reagents was kept constant. The second assay measured dilutions of all the reaction components to find out the minimum amounts needed for a reliable assay. The results revealed that screening assay costs will be lower with EnVision compared to the other instruments tested because it enables use of lower amounts of reaction components. Considering these low costs in combination with its rapid plate measurement, the Envision is a very attractive instrument for routine use in a screening laboratory. The most sensitive instrument for phosphopeptide measurements with black microplates was also EnVision, but VICTOR outperformed EnVision when white microplates were used.

TruPoint is an assay method based on time-resolved fluorescence quenching and has been developed to measure activity of hydrolyzing enzymes. With the TruPoint assay, use of white microplates resulted in better sensitivity than black ones with all of the instruments. Thus in applications measuring low enzyme activities, such as measurement of protease activity from cell lysates, the use of white microplates is recommended. There were no significant differences in plates of different densities indicating that TruPoint assays developed in 96- or 384-well formats are easily miniaturizable into the 1536-well format. The white microplates tested were not compatible with the Fusion in the TruPoint assay as the PMT of the instrument became saturated with the wells having high enzyme concentrations. The Z' values were comparable with all of the instruments. Even though the Z' values were lower with black microplates compared to the white ones, the use of black microplates is recommended for HTS applications. The black microplates have better tolerance for library compounds absorbing at the europium emission wavelength thus reducing the number of false positives in HTS.

Microplate reading times were also compared for the different instruments. The reading times with ViewLux were clearly shortest due to the imaging technology used. EnVision outperformed VICTOR and Fusion, especially in LANCE assays where EnVision utilized a dual measurement mode which is not available for VICTOR or Fusion. Both EnVision and ViewLux are extremely fast plate readers for high throughput screening, while VICTOR and Fusion are better suited for research and assay development purposes.

## Available Products

| Product No. | Description |
|---|---|
| 6005290 | OptiPlate-96 (96-well white microplate, 50 plates) |
| 6005270 | Optiplate-96 F (96-well black microplate, 50 plates) |
| 6007290 | OptiPlate-384 (384-well white microplate, 50 plates) |
| 6007270 | OptiPlate-384 F (384-well black microplate, 50 plates) |
| 6005228 | OptiPlate-1536 (1536-well white opaque microplate, 20 plates) |
| 6005235 | OptiPlate-1536 F (1536-well black microplate, 20 plates) |
| AAAND-0001 | DELFIA Yellow Plate (96-well microplate, 60 plates) |
| AD0121<br>*Includes:* | LANCE Tyrosine Kinase Start Up Reagents<br>AD066 (50 µg) LANCE Eu-W1024 anti-pTyr antibody (PY100)<br>AD067 (1 mg) LANCE Eu-W1024 anti-pTyr antibody (PY100)<br>CR130-100 (1 mg) SureLight APC-Streptavidin<br>CR130-150 (50 mg) SureLight APC-Streptavidin<br>Biotinylated poly(Glu, Tyr) kinase substrate<br>Biotinylated phosphotyrosine peptide (positive control)<br>6007290 OptiPlate-384 white |
| 1244-360 | DELFIA Eu-labeled Streptavidin, 250 µg |
| B119-100 | DELFIA 1 nmol/L Europium Standard Solution, 50 mL |
| AD0125 | TruPoint Caspase-3 Assay Kit, 2 plates |
| 2101 | EnVision Multilabel Plate Reader |
| 1420 | VICTOR$^2$V Multilabel Plate Reader |
| 1430 | ViewLux Multilabel Plate Imager |
| *Not available* | Fusion Multilabel Plate Reader |

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