

# Using Polypropylene Microplates in Luminescence and Fluorescence based Biochemical Assays

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## Introduction

Luminescence and Fluorescence are widely used, highly sensitive, non radioactive detection methods for monitoring chemical reactions on a biochemical and cellular level (Fig. 1).

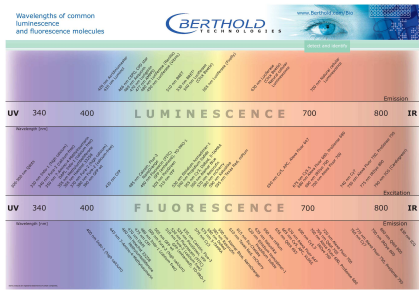


Fig. 1: Wavelengths of common fluorescence and luminescence molecules

- Polystyrene (PS) microplates (Fig. 2) are frequently used in drug discovery as they are available with a wide range of surface treatments which renders them especially beneficial for cell based assays.
- However for common biochemical homogenous assays, polypropylene (PP) may demonstrate advantages over PS due to inherent physical properties that result in reduced biomolecular binding capacities, higher temperature tolerance, increased chemical resistance and increased physical stability (Data not shown).
- Recently a new 384 Deep Well Small Volume™ microplate (Fig. 3) was introduced for direct compound transfer with pin tools. Although this new microplate was chiefly developed for compound storage, it was additionally conceptualized at the onset with awareness of a requirement for black and white pigmented 384 well Deep Well Small Volume™ microplates in fluorescence and luminescence measurements.

## Microplates / Assay / Detection

# 781075: 384 Well, Polystyrene, White  
# 781076: 384 Well, Polystyrene, Black

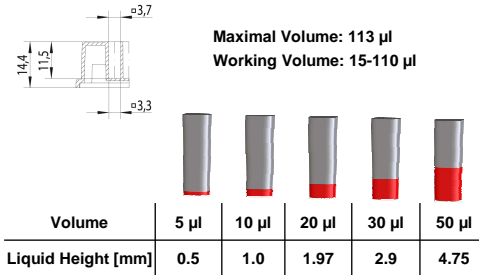


Fig. 2: 384 Well PS microplate. Well geometry and liquid height depending on assay volume

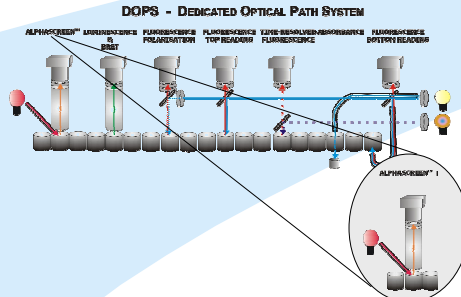


Fig. 4: Luminescence and fluorescence readouts were done with the Mithras LB 940 microplate reader (Berthold Technologies, Bad Wildbad, Germany)

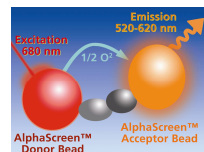


Fig. 5: The focus of the performed tests were fluorescence measurements and the Amplified Luminescence Proximity Assay Technology (ALPHA Screen™, Perkin Elmer, Shelton, CT, USA)

# 784207: 384 Deep Well, SV, Polypropylene, White  
# 784209: 384 Deep Well, SV, Polypropylene, Black

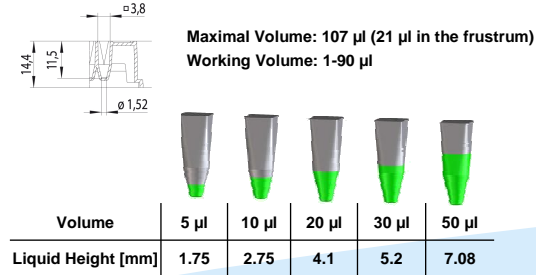


Fig. 3: 384 Deep Well PP SV microplate. Well geometry and liquid height depending on assay volume

## Results and Discussion

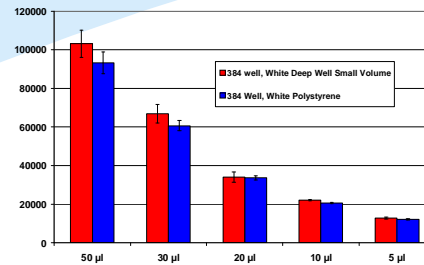


Fig. 6: ALPHA Screen™: Signal strength depending on assay volume

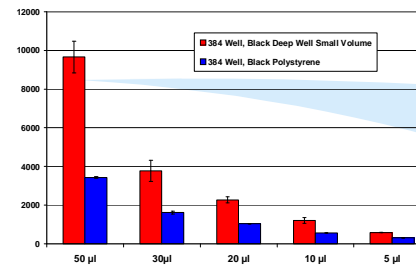


Fig. 7: Measurement of Fluorescein (8.85 x 10<sup>-5</sup> mM). Signal strength depending on assay volume

	Length	Width	A1	A1
	127.76	85.48	12.13	8.99
781 076: PS Black	127.68	85.44	12.15	9.01
781 075: PS White	127.69	85.48	12.19	8.97
784 209: SV, PP, Black	127.75	85.59	12.07	9.07
784 207: SV, PP, White	127.69	85.58	12.09	9.04
ANSI Tolerances	+/- 0.25	+/- 0.25	N.D.	N.D.

Tab. 1: Dimensions of 384 Well PS and 384 PP Deep Well Small Volume™ microplates. Measurement of three microplates / reference)

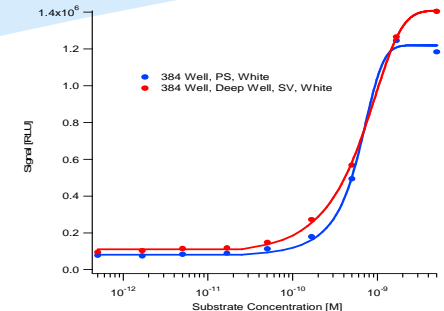


Fig. 8: Alpha Screen Fluorescein detection kit. Dilution of biotinylated Fluorescein as substrate

- For luminescence signal detection in the ALPHA Screen™ the Deep Well PP SV™ plate performed slightly better than the PS plate (Fig. 6, Fig. 8). Depending on the signal strength and assay sensitivity this difference can be advantageous.
- In fluorescence signal detection (Fig. 7) the 384 Deep Well PP SV™ plate performed much better.
- The truncated cone of the Deep Well PP SV™ plate results in higher liquid levels enabling major savings of reagents (Fig. 2, Fig 3).
- Identical plate dimensions with tight tolerances (Tab. 1) enable the use of a single plate editor and allow reliable signal detection.
- The Mithras plate reader (Fig. 4) features both: flexible detection and compatibility to all plate formats from 96 to 1536 wells.