

Labrox multimode reader featuring Upcon™ technology

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Introduction

Upcon™ is a comprehensive concept built around a novel label technology known as photon upconversion. In this unique technology, upconverting nanoparticles (UCNPs) convert near-infrared (NIR) radiation to visible light. Due to the ability of the UCNPs to absorb two sequential NIR photons and subsequently emit one high-energy photon as visible light, which is measured, the Upcon technique is extremely efficient in eliminating autofluorescence in biological samples. UCNPs produce bright luminescence with discrete emission bands and long emission lifetime (μs range). NIR excitation and emission reading at 660 nm that are within the optical window (600-1200 nm) make them detectable through tissue and even in whole blood.

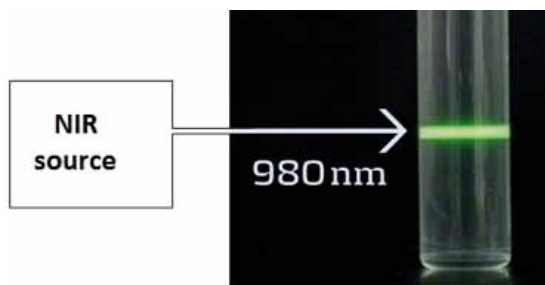


Figure 1. Exceptional NIR-excitable label particles: absorption of two NIR photons is followed by emission of one high-energy photon at visible wavelengths

The Upcon concept is formed by the UCNPs (produced by Kaivogen Oy) and by the first commercial microplate reader dedicated for use with UCNPs, the Labrox Upcon reader.

In this application note, we demonstrate the functioning of the Upcon technology in the Labrox readers.



Figure 2. Labrox Upcon plate reader.

Materials

- Kaivogen Upcon™ nanoparticles, Erbium-540-COOH, functional group: carboxylic acid (43-08RD) (coated particles)
- Kaivogen Upcon nanoparticles, Erbium-540-core, non-functionalized (40-28RD) (Core particles)
- transparent plates (PerkinElmer SpectraPlate™-96 MB)
- white plates (PerkinElmer, OptiPlate™-F 96)
- 0,1 M Na-acetate buffer pH 4.0 + 0,1% Tween 20 (AB)
- 20 mM borate buffer pH 8.0 (BB)
- pipettes and tips
- tubes
- vortex
- Labrox reader with Upcon mode

Methods

Dilution series of the core and coated UCNPs particles were prepared in two different buffers: 0,1 M Na-acetate buffer pH 4.0 (AB) and 20 mM borate buffer pH 8.0 (BB), as shown in Table 1.

Table 1. Dilution series of the core and coated UCNPs in AB or BB.

Core or coated [UCNPs] (pg/ml)	Vol. of stock (5 mg/ml)	AB or BB	Vol. (μl)	Vol. final (μl)
① 1000000	30 μl	1470	1500	1350
② 100000	150 μl ①	1350	1500	1350
③ 10000	150 μl ②	1350	1500	1350
④ 1000	150 μl ③	1350	1500	1350
⑤ 100	150 μl ④	1350	1500	1350
⑥ 10	150 μl ⑤	1350	1500	1350
⑦ 1	150 μl ⑥	1500	1500	1500

Experiments in both white and transparent plates were performed. Six replicates of 100 μl were added to the appropriate wells in the plates and immediately measured.

Core particles

in AB

- white plate
- transparent plate

Coated particles

in AB

- white plate
- transparent plate

in BB

- white plate
- transparent plate

Figure 3. Diagram showing the performed experiments.

Measurement conditions:

Upcon mode, Endpoint
EMS Filter UPCON 540 nm
Em EMS SPOT size: 4.0 mm
Z-focus: 5 mm

Two different measuring times (10 and 100 ms) and two different laser powers (10 and 30%, corresponding to 0,1 and 0,3 W respectively) were used for the measurements, in order to determine the best measurement parameters.

The sensitivity - LoD (Limit of Detection or the lowest quantity of a substance that can be distinguished from the absence of that substance) was calculated according to IUPAC standards:

$$\text{LoD} = 3 \cdot \text{SD}_{\text{blank}} / \text{slope}_{\text{standard curve}}$$

LoD was determined for the different measuring conditions tested in this application note (Fig. 10).

Results

NOTE: All the data used to draw the following presented figures are available upon request. Presenting here a large amount of data would make this application note too extensive.

Core particles

Transparent plates: the best parameters for Upcon measurements in these conditions were 100 ms and 10 % laser power. (Figures 4 and 10).

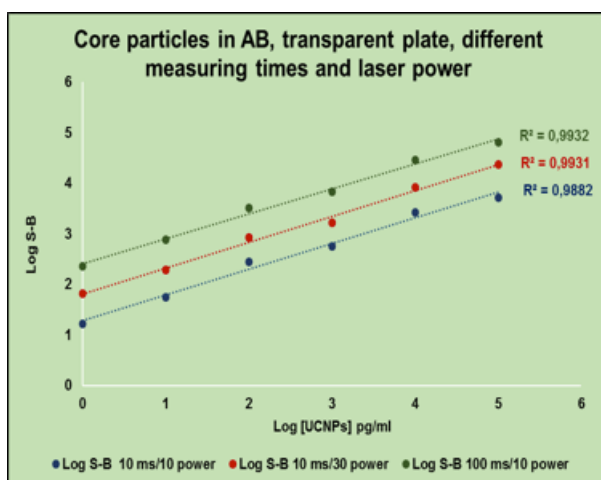


Figure 4. Logarithm of the S-B (signal minus background) ratios plotted against logarithm of [core UCNPs] measured in AB in transparent plates under different measuring parameters.

White plates: the best measurement conditions were also in this case 100 ms and 10% power (Figures 5 and 10).

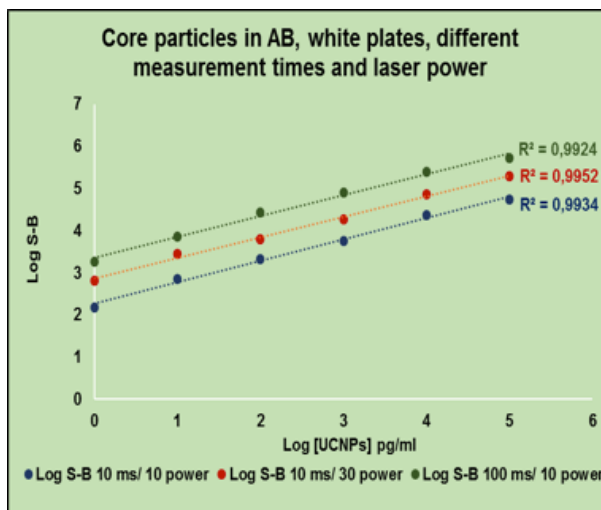


Figure 5. Logarithm of the S-B ratios plotted against logarithm of [core UCNPs] measured in AB in white plates under different measuring parameters.

The signal in white plates was always higher and the LoD lower than in transparent plates under the same measurement parameters.

Coated particles

A. In AB, transparent plates: the best parameters for Upcon measurements in these conditions were 100 ms and 30 % power (Figures 6 and 10).

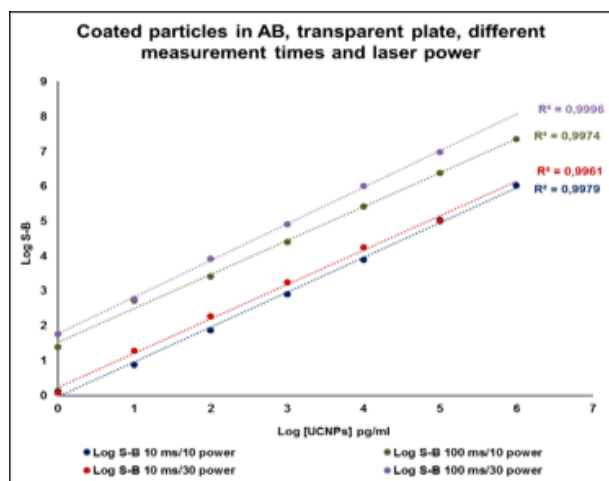


Figure 6. Logarithm of the S-B ratios plotted against logarithm of [coated UCNP] measured in AB in transparent plates under different measuring parameters.

B. In AB, white plates: the best parameters for Upcon measurements in these conditions were 100 ms and 30 % power (Figures 7 and 10).

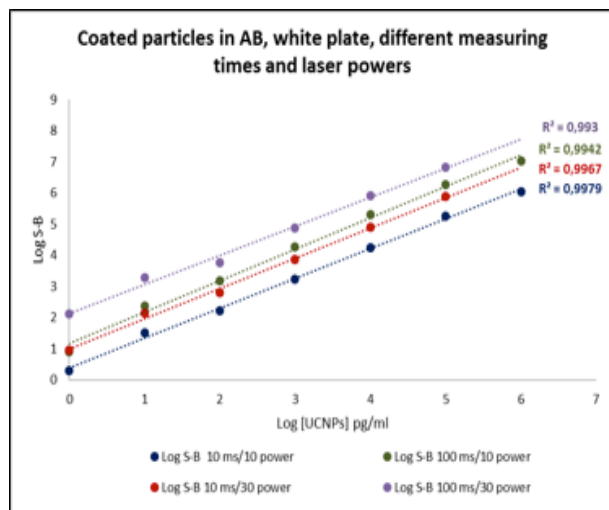


Figure 7. Logarithm of the S-B ratios plotted against logarithm of [coated UCNP] measured in AB in white plates under different measuring parameters.

The signal generated by the coated UCNP in AB was always higher in white plates than in transparent plates under the same measurement parameters.

C. In BB, transparent plates: the best parameters for Upcon measurements in these conditions were 100 ms and 30 % power (Figures 8 and 10)

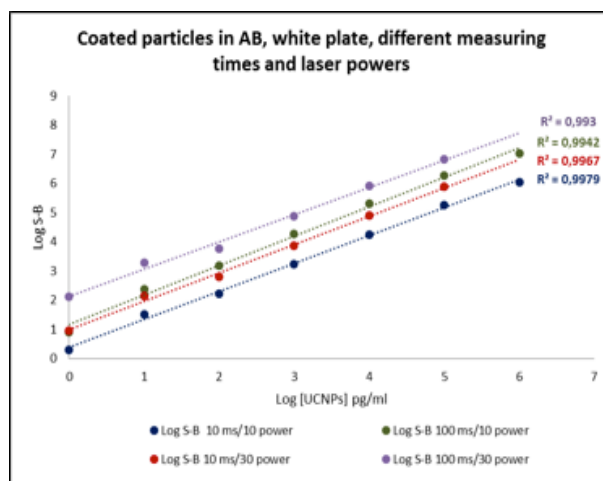


Figure 8. Logarithm of the S-B ratios plotted against logarithm of [coated UCNP] measured in BB in transparent plates under different measuring parameters.

D. In BB, white plates: the best parameters for Upcon measurements in these conditions were 100 ms and 30 % power (Figures 8 and 10).

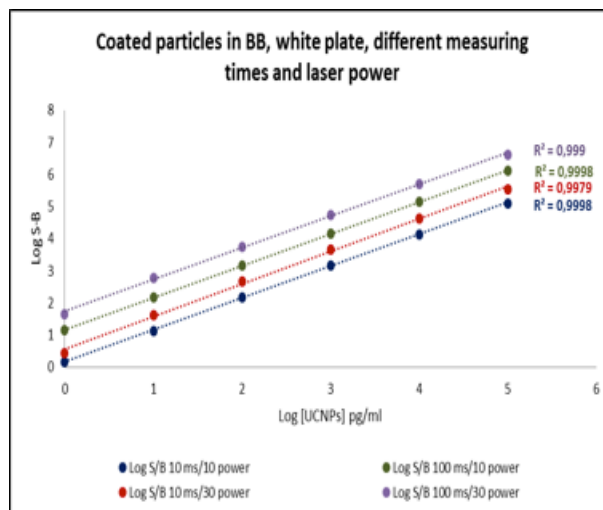


Figure 9. Logarithm of the S-B ratios plotted against logarithm of [coated UCNP] measured in BB in white plates under different measuring parameters.

Coated UCNP in BB also generated higher signal in white plates than in transparent plates under the same measuring parameters.

Figure 10 shows the LoDs calculated for all the performed measurements:

	ms/power	LoD (pg/ml)		
Core particles	in AB	transp. plate 10/10	18,96	
		transp. plate 10/30	5,34	
		transp. plate 100/10	3,1	
	white plate	10/10	7,62	
		10/30	NA	
		100/10	3,83	
Coated particles	in AB	transp. plate 10/10	9,04	
		transp. plate 10/30	7,29	
		transp. plate 100/30	0,39	
	white plate	10/10	1,5	
		10/30	0,844	
		100/30	0,37	
	in BB	transp. plate	10/10	13,32
			10/30	4,8
			100/30	0,94
white plate		10/10	3,54	
		10/30	1,11	
		100/30	0,31	

Figure 10. LoDs with both core and coated UCNP, in different measuring conditions.

Conclusions

- Labrox Upcon plate reader can accurately measure UCNP, both core and coated particles.
- The Labrox Upcon reader not only provides extremely sensitive measurements of UCNP (LoDs <1 pg/ml under certain parameters) but also the possibility of improving the sensitivity levels by changing the measuring parameters.
- Both 0,1 M Na-acetate buffer pH 4.0 + 0,1% Tween 20 and 20 mM borate buffer pH 8.0 are good buffers to use in Upcon measurements.
- LoD calculation in the different measuring conditions showed very clearly that:

1. Sensitivity is higher in white plates than in transparent plates with both core and coated UCNP and in both AB and BB (e.g core particles have a LoD of 18,96 at 10/10 in transparent plates and a LoD of 7,62 in white plates, under the same measurement parameters)

2. The increase of measurement time and/or laser power increases the sensitivity of the technique too, reducing the LoD.

- Even we recommend the use of white plates when possible, these can easily be replaced by transparent ones in case of need: by using higher reading times and/or higher laser power the results can be highly improved in transparent plates.

References

1. www.upcon.fi/
2. www.iupac.org/