

Application Note 10

Measurement of Estrogen Receptor- β Assay with LF502NanoScan FLT

measurement date: 26/04/2007

Aim of the experiment:

- evaluation of an Estrogen Receptor- β Competitor Assay (Invitrogen Corporation, Carlsbad, CA, USA; Part # P2615, P2700) for fluorescence lifetime read-out
- investigation of the influence of the number of averaged laser-pulses on the z' -value
- comparison of two different methods of numerical curve analysis on z' -value



Sample preparation:

concentration values of stock solutions:

Fluoromon (green): 400 nM
 Estrogen Receptor: 4,5 μ M
 Estradiol in Ethanol: 75 mM

sample preparation in a black 384 well plate (Greiner Fluotrac 200): 50 μ L per Well

preparation of the 2X complex with Fluoromon (2nM) and receptor (20nM) (according to Invitrogen protocol for 70 wells x 25 μ L):

Fluoromon: $(70 \cdot 25 \mu\text{L} \cdot 0,002 \text{pMol}/\mu\text{L}) / 0,4 \text{pMol}/\mu\text{L} = 8,75 \mu\text{L}$

receptor: $(70 \cdot 25 \mu\text{L} \cdot 0,02 \text{pMol}/\mu\text{L}) / 4,5 \text{pMol}/\mu\text{L} = 7,78 \mu\text{L}$

in total for 2X complex: 1733,5 μ L buffer + 8,75 μ L Fluoromon + 7,78 μ L receptor

preparation of Estradiol standard concentrations in the plate with 25 μ L per well:

dilution of the Estradiol stock solution: two times 1:100 => c = 7,5 μ M

then 1:7,5 => stock solution with 1000 nM Estradiol

then 1:10 => stock solution with 750 nM Estradiol (for low control)

Now

- pipetting of 25 μ L screening buffer in 3 columns of the 384 well plate (columns 11 ..13; row A to L) and
- pipetting of 25 μ L screening buffer in column 14 (for high control)
- pipetting of 25 μ L 750-nM-Estradiol stock solution in column 15 (for low control)

addition of 25 μ L 1000-nM-Estradiol stock solution to A11, A12 and A13

then preparation of 1:2 dilution steps in the plate (transfer of 25 μ L from row A to B, then from B to C, ...)

last step: addition of 25 μ L 2X complex to all 68 wells

layout with final Estradiol concentrations:

	11	12	13	14	15	
A	500	500	500	high	low	
B	250	250	250	high	low	
C	125	125	125	high	low	
D	62,5	62,5	62,5	high	low	
E	31,25	31,25	31,25	high	low	
F	15,63	15,63	15,63	high	low	
G	7,81	7,81	7,81	high	low	
H	3,91	3,91	3,91	high	low	
I	1,95	1,95	1,95	high	low	
J	0,98	0,98	0,98	high	low	
K	0,49	0,49	0,49	high	low	
L	0,24	0,24	0,24	high	low	
M				high	low	
N				high	low	
O				high	low	
P				high	low	

final Estradiol concentration of low control: 375 nM

incubation: 2h

Instrument settings for Fluorescence lifetime measurements:

filter settings:

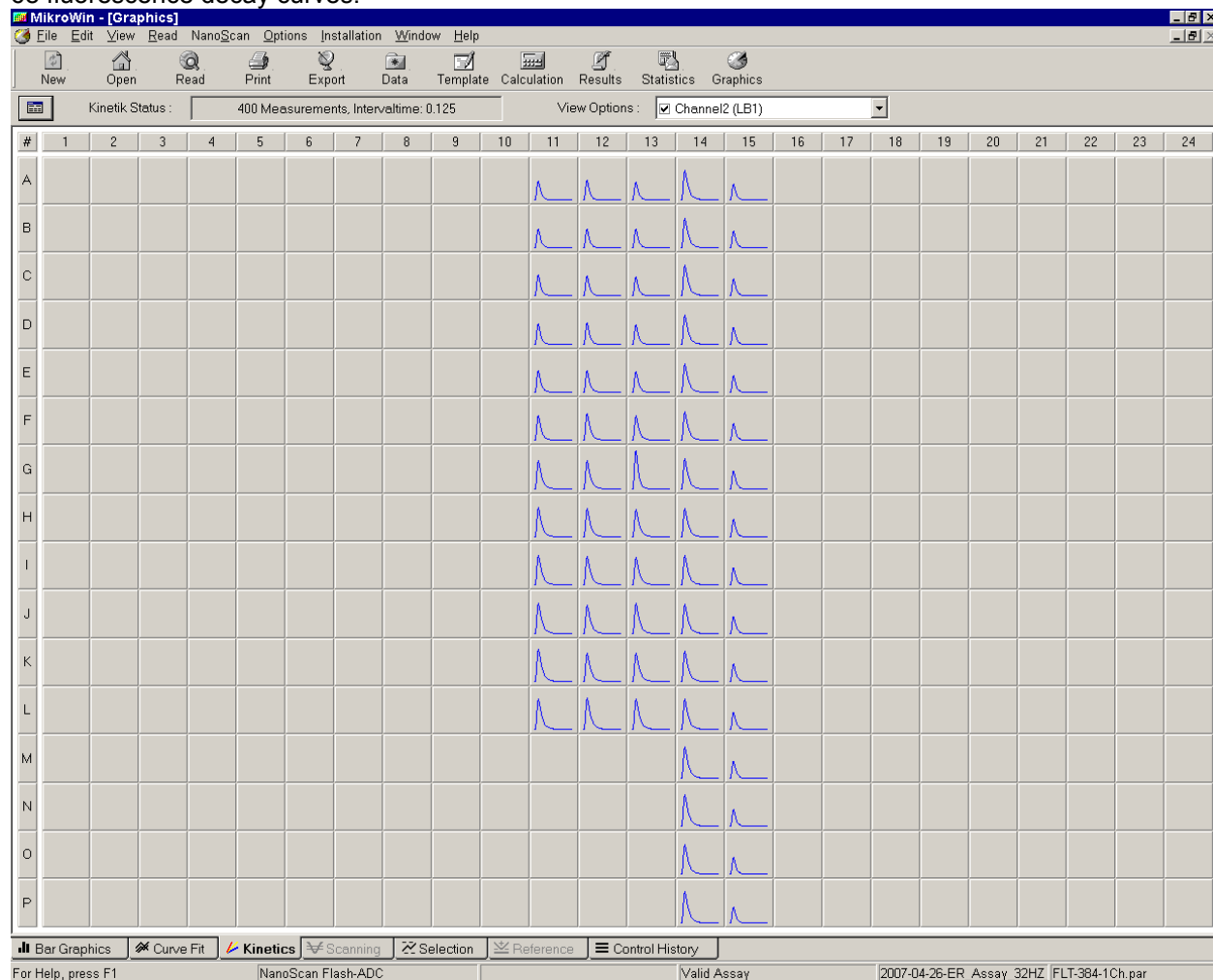
filter cube:	excitation:	488/10
	beam splitter:	505 DCLP
	emission:	520/10
laser frequency:	20 Hz	
laser energy:	20 %	
gain Channel 2:	reduced gain	
measurement window:	50 ns	

the number of laser pulses per well to be averaged over was varied between 1 and 128 in order to investigate the influence on the z' value

Results:

Fluorescence lifetime measurements

68 fluorescence decay curves:



numerical curve analysis:

fitting range:

number of exponentials:

mode of operation:

complete fluorescence decay curve including rising edge

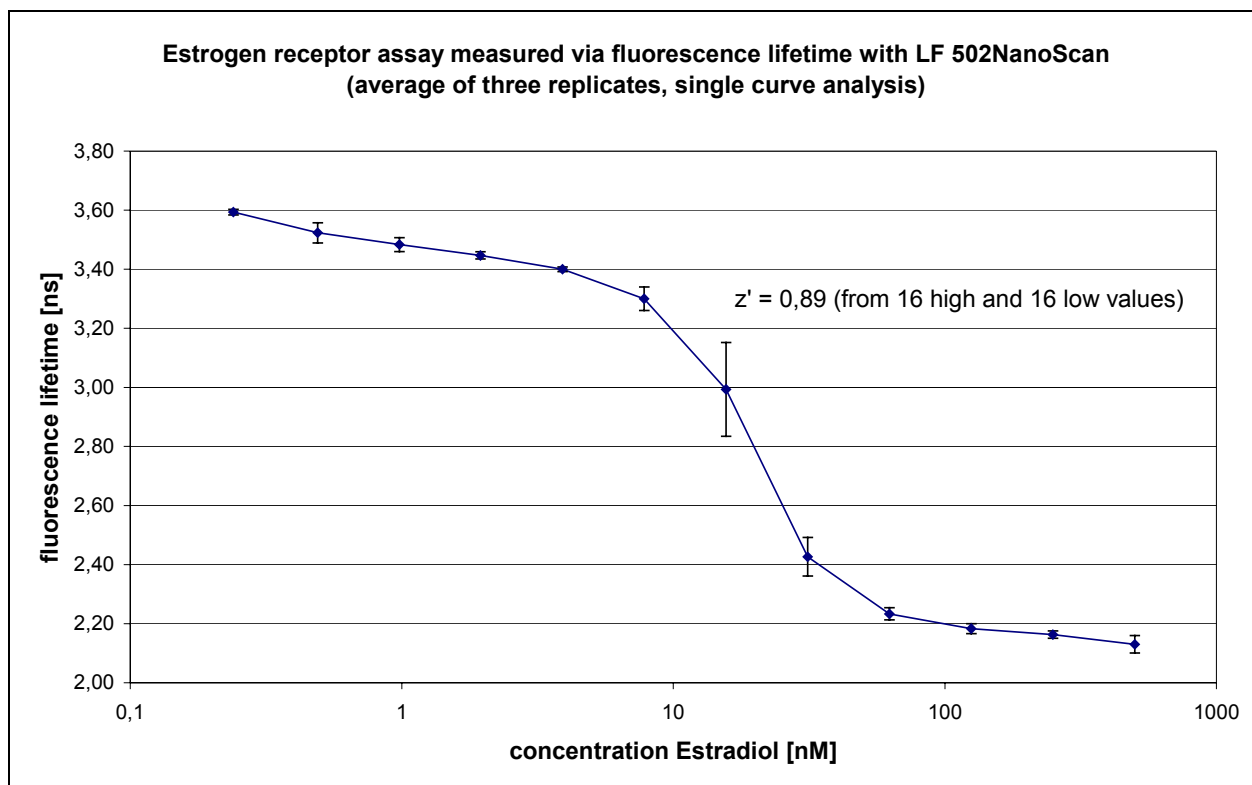
single-exponential

automatic single curve analysis

averaged number of laser pulses per well	z'-value
1	0,57
2	0,74
4	0,87
8	0,86
32	0,89
128	0,92

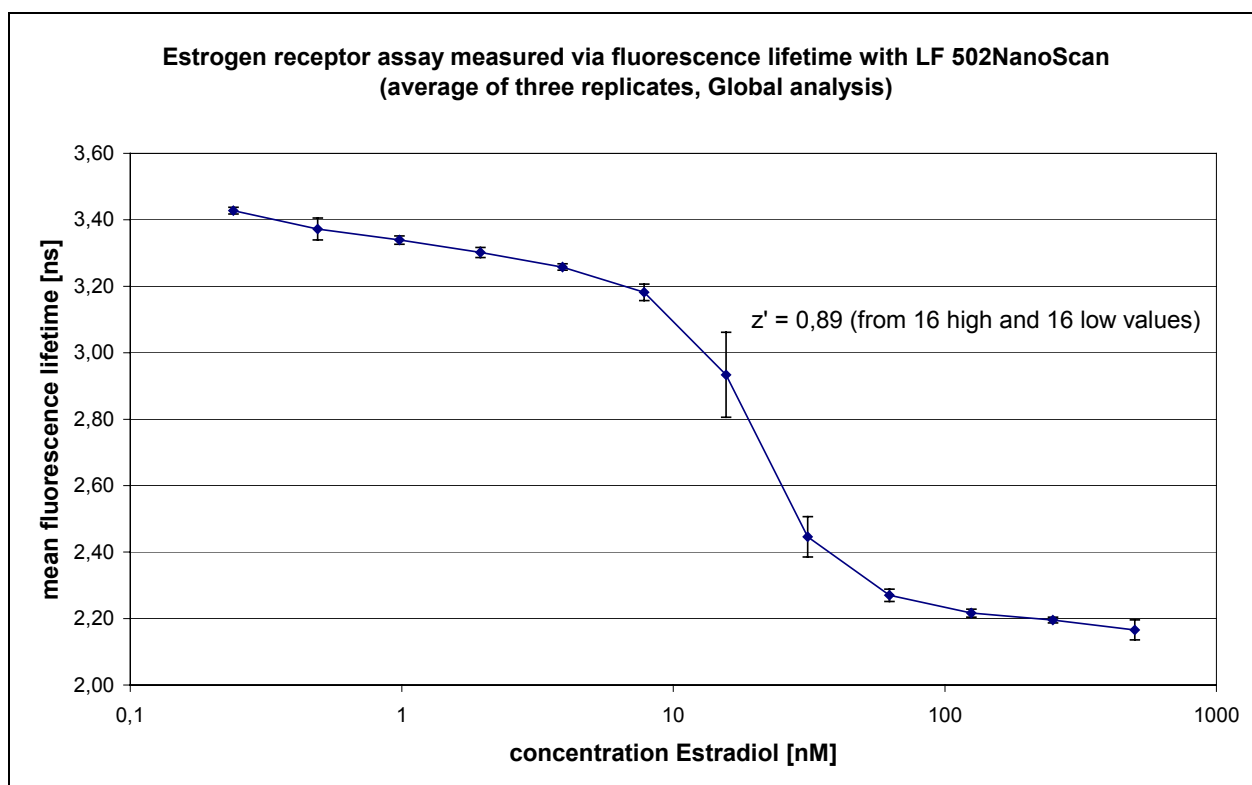
comparison of different methods of numerical curve analysis on standard curves:

derived from the measurement with 32 laser pulses per well – **single curve analysis** (see above)



derived from the measurement with 32 laser pulses per well – **Global analysis**:

fitting range:	complete fluorescence decay curve including rising edge
number of exponentials:	double-exponential
output result:	mean fluorescence lifetime



Conclusion:

- The Estrogen receptor- β assay is easily readable using fluorescence lifetime mode.
- High z' values are achieved with only 4 to 8 laser pulses per well.
- Single curve analysis (fast method) with single-exponential model is sufficient for high statistical data quality.
- Global analysis with a double-exponential model (physically correct, slower method) yields the same level of z' -values and slightly better standard curves.