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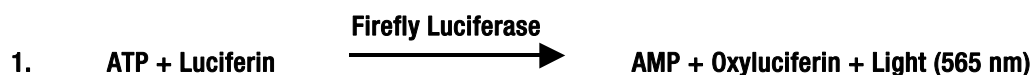
Orion II Microplate Luminometer Simplicity 4

Luciferase Reporter Assay

Dual Reporter genes are widely used to study gene expression and regulation mechanisms in living cells. Not all expressed enzymes are easily detectable, so reporter genes were introduced into cellular DNA to investigate gene function by means of a measurable property, the luminescence.

The most popular reporter is the firefly luciferase from the American firefly (*Photinus pyralis*). The high sensitivity, easy handling, short process time and a high quantum yield of the bioluminescence reaction make this method to the "method of choice" in controlling gene expression. The assay used in this note is optimized for extended half life time of more than five minutes.

Reaction



Materials

Luminometer: Orion II Microplate Luminometer with 2 injectors
Software: Simplicity 4
Assay : Example for a commercial Luciferase Assay
Microplates: opaque microplates (solid, white, 96 well), No: 3912, supplied by Corning or Nunc No: 236108

Method

Usually 20-100 µl aliquots of lysates or standards of purified Firefly Luciferase were put in the wells as triplicates.

The Lysis Buffer was diluted according to the assay description with deionized or distilled water.

In the described assay the injection lines were prepared by priming injector 1 with substrate A (ATP) and injector 2 with substrate B (Luciferin). Automatic reagent injectors were programmed to dispense 100µl of each reagent.

Delay between the injections: 2s

Delay between second injection and measurement: 2s

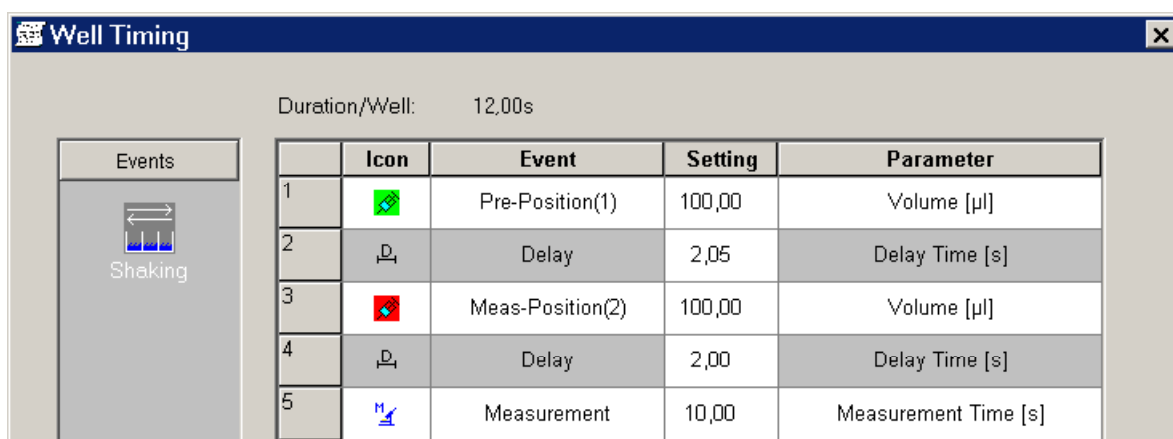
Measuring Time: 5-10 s

→ see the instruction sheet of the used Luciferase Assay Kit !






PC-Settings

- ◆ Create a *Raw Data* protocol.
- ◆ Select the microplate format for 96 wells.
- ◆ Define your well timing (see an example in the screenshot below)
- ◆ Select the wells you want to measure or choose whole plate.
- ◆ Select if you want to measure the background, and if you want to save automatically.
- ◆ Decide if you want an automatic Excel Transfer and save the protocol

Event Table in the Raw Data Protocol



Duration/Well: 12,00s

	Icon	Event	Setting	Parameter
1		Pre-Position(1)	100,00	Volume [µl]
2		Delay	2,05	Delay Time [s]
3		Meas-Position(2)	100,00	Volume [µl]
4		Delay	2,00	Delay Time [s]
5		Measurement	10,00	Measurement Time [s]

The screenshot shows standard settings of a usual protocol of a commercial Luciferase Assay.

Example

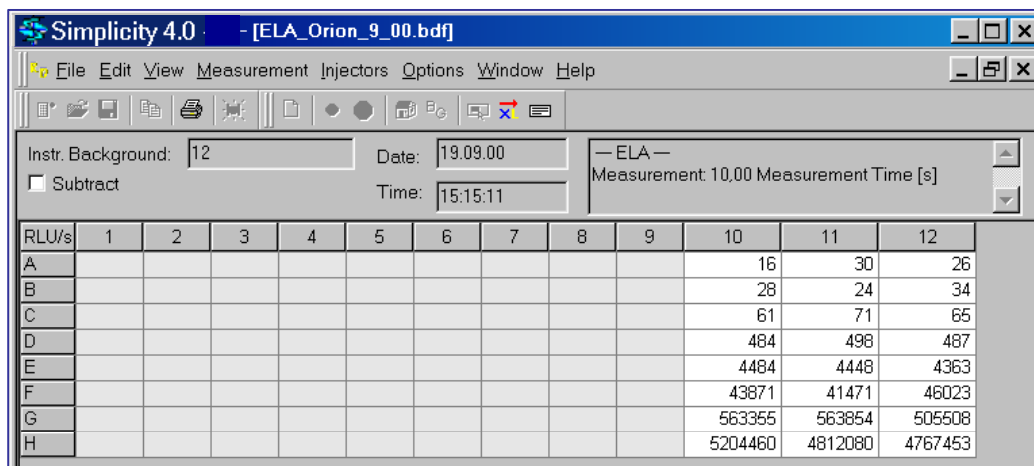
Measurement of different dilutions of a stock solution containing purified luciferase.
 20µl were added in the wells as triplicates.

- A 10-12: 3 replicates of lysis buffer (chemical background)
- B 10-12: 3 replicates of 10⁻⁸ dilution
- C 10-12: 3 replicates of 10⁻⁷ dilution
- D 10-12: 3 replicates of 10⁻⁶ dilution
- E 10-12: 3 replicates of 10⁻⁵ dilution
- F 10-12: 3 replicates of 10⁻⁴ dilution
- G 10-12: 3 replicates of 10⁻³ dilution
- H 10-12: 3 replicates of 10⁻² dilution

Application Note 2004/03 Orion II Microplate Luminometer Simplicity 4

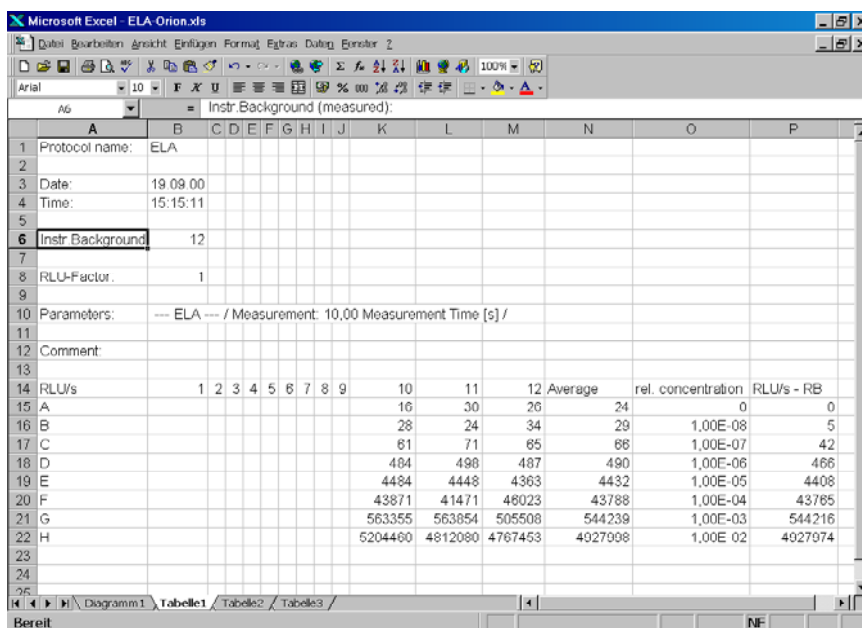
Measurement Results

In the measurement window the data are displayed as raw data in RLU/s.



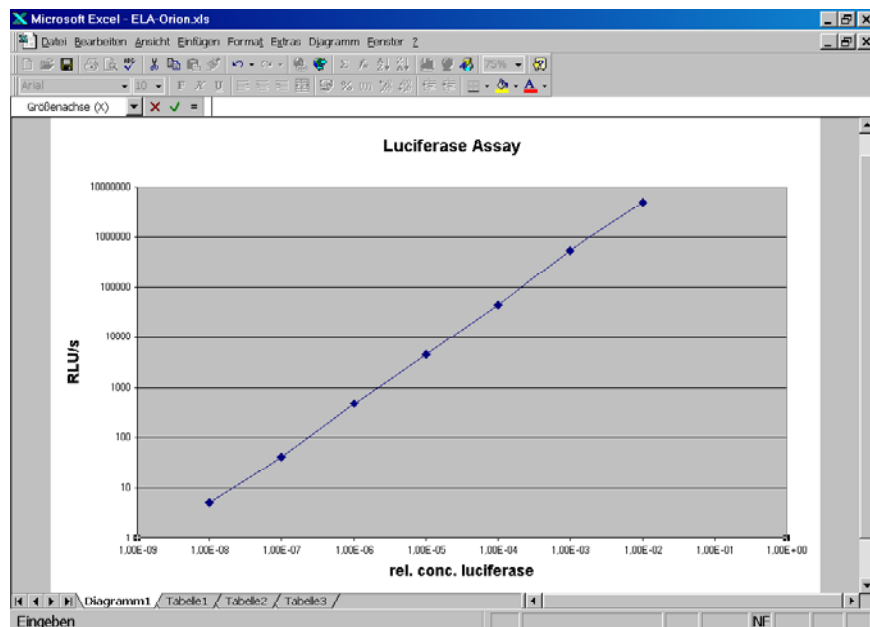
RLU/s	1	2	3	4	5	6	7	8	9	10	11	12
A										16	30	26
B										28	24	34
C										61	71	65
D										484	498	487
E										4484	4448	4363
F										43871	41471	46023
G										563355	563854	505508
H										5204460	4812080	4767453

Transfer to Microsoft® Excel by clicking the XL-Button



RLU/s	1	2	3	4	5	6	7	8	9	10	11	12	Average	rel. concentration	RLU/s - RB
A										16	30	26	24	1,00E-08	0
B										28	24	34	29	1,00E-08	5
C										61	71	65	66	1,00E-07	42
D										484	498	487	490	1,00E-06	466
E										4484	4448	4363	4432	1,00E-05	4408
F										43871	41471	48023	43788	1,00E-04	43785
G										563355	563854	505508	544239	1,00E-03	544216
H										5204460	4812080	4767453	4927998	1,00E-02	4927974

Microsoft® Excel Graph



Discussion:

The relative light units per second (RLU/s) were plotted against the relative concentration of the Firefly Luciferase. All RLU/s were calculated by subtracting the background signal of the reagent blank (wells A10-A12). The signal is linear over six decades of enzyme concentration and shows the high dynamic range of the instrument.