
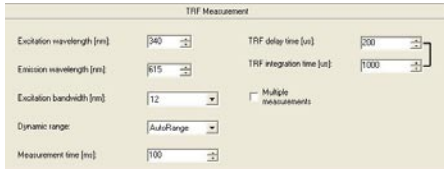


TRF measurements with Varioskan®



Varioskan is a spectral scanning multimode reader capable of photometric, fluorometric and time-resolved fluorometric measurements. This sheet will give a short description how to perform different time-resolved fluorometric (TRF) assays with Varioskan.

Time-resolved fluorescence means that the measurement of the specific fluorescence emission signal is started after a delay. During the delay the short-lived background fluorescence has decayed. The following table contains a short description of each TRF measurement type. The parameters given on the table are only the default values for Europium and Europium-APC pair and need to be determined specifically for each assay.

Measurement type	Measurement parameter set	Example of result																																				
<p>TRF measurement (endpoint)</p>  <p>The measurement is used for determining the amount of lanthanide label in the sample as in a fluorescence intensity measurement.</p>	<p>The following parameters (in minimum) need to be defined:</p> 	<p>The result is relative to the label concentration. The result is given as RFU (relative fluorescence unit)</p> <table border="1"> <thead> <tr> <th>Pl</th> <th>Well</th> <th>Sample</th> <th>RFU</th> </tr> </thead> <tbody> <tr> <td>1</td> <td>A01</td> <td>Un_0001 1/1</td> <td>74.9229</td> </tr> <tr> <td>1</td> <td>A02</td> <td>Un_0017 1/1</td> <td>457.015</td> </tr> <tr> <td>1</td> <td>A03</td> <td>Un_0033 1/1</td> <td>1822.26</td> </tr> <tr> <td>1</td> <td>A04</td> <td>Un_0049 1/1</td> <td>736.564</td> </tr> <tr> <td>1</td> <td>A05</td> <td>Un_0065 1/1</td> <td>829.493</td> </tr> <tr> <td>1</td> <td>A06</td> <td>Un_0081 1/1</td> <td>4342.63</td> </tr> <tr> <td>1</td> <td>A07</td> <td>Un_0097 1/1</td> <td>2228.46</td> </tr> <tr> <td>1</td> <td>A08</td> <td>Un_0113 1/1</td> <td>595.416</td> </tr> </tbody> </table>	Pl	Well	Sample	RFU	1	A01	Un_0001 1/1	74.9229	1	A02	Un_0017 1/1	457.015	1	A03	Un_0033 1/1	1822.26	1	A04	Un_0049 1/1	736.564	1	A05	Un_0065 1/1	829.493	1	A06	Un_0081 1/1	4342.63	1	A07	Un_0097 1/1	2228.46	1	A08	Un_0113 1/1	595.416
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TRF Scanning



With scanning measurement both excitation and emission spectra can be created.
By analyzing the spectra data the optimal parameters e.g. excitation and emission wavelengths can be chosen for each assay.

Excitation spectrum parameters

TRF Scanning

Fixed wavelength: []

Excitation: Emission:

Wavelength [nm]: [615]

TRF delay time [μs]: [200]

TRF integration time [μs]: [1000]

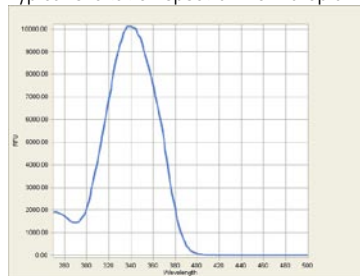
Scanning wavelengths:

Start [nm]: [300]

End [nm]: [500]

Step size [nm]: [1]

Typical excitation spectrum for Europium



Emission spectrum parameters

TRF Scanning

Fixed wavelength: []

Excitation: Emission:

Wavelength [nm]: [615]

TRF delay time [μs]: [200]

TRF integration time [μs]: [1000]

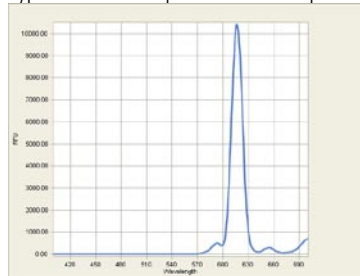
Scanning wavelengths:

Start [nm]: [400]

End [nm]: [700]

Step size [nm]: [1]

Typical emission spectrum for Europium



TRF decay



Fluorescence lifetime (τ) means the average interval between absorption and emission actions.
 τ is the time where approximately 67% of the signal has decayed.
The decay data can be used for optimization of measurement parameters

TRF Decay

Excitation wavelength [nm]: [340]

Emission wavelength [nm]: [615]

Excitation bandwidth [nm]: [12]

Dynamic range: [AutoRange]

Measurement time [ms]: [100]

Time Setting

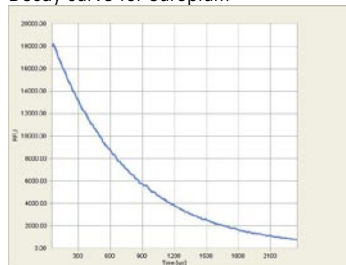
Start time [μs]: [50] [Edit...]

Stop time [μs]: [2350]

Step [μs]: [10]

Integration time [μs]: [100]

Decay curve for europium



Calculated values for the following parameters are also reported: τ , α (theoretical maximum signal), recommended integration time and R^2

TR-FRET



An energy transfer measurement always contains two different labels. The measurement may be a one endpoint measurement or may contain the measurement of both of the labels separately. The assay type is used for homogeneous screening assays e.g. LANCE

TRF Measurement

Excitation wavelength [nm]: [340]

Emission wavelength [nm]: [615]

Excitation bandwidth [nm]: [12]

Dynamic range: [AutoRange]

Measurement time [ms]: [100]

Log time [hours:ms]: [00:00:00.00]

Stop duration [hours:ms]: [00:00:00.00]

Measurement interval [hours:ms]: [00:00:00.00]

Multiple measurements

Ex	Emiss	Delay time [μs]	Integration time [μs]
340	615	200	1000
300	605	200	1000

The result is relative to the label concentration. The result is given as RFU (relative fluorescence unit)

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