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Plate reader setting

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Protocol status: Working
We use this protocol and it's working

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Abstract

A brief guideline of setting the plate reader to measure the sfGFP production.

Guidelines

This protocol is a supplemental guideline for Toehold functionality test, it is developed based on PerkinElmer VICTOR X3, certain terminologies might vary from different equipments, please check the instruction of your own plate reader when some of the steps are unclear.

1 Create a new protocol in PerkinElmer by clicking **start wizard** in Victor Workstation. Name it as the substance you want to measure. In our case sfGFP, select a folder to save the protocol. Click **Next>**.

2 Select the plate you are using for the measurement, it allows the plate reader to have a default setting of the position of each well according to the plate you chose, we used **NUNC 384**. Click **Next>.**

3 Select or create the label we are using for the measurement, the label is usually named as the substance you are testing it gives setting for lamp, filter and counting time for the measurement.
Click **Label** (the fourth icon)on the right navigation bar to add labels and search in **Fluorometry** tab to see if there is already existing sfGFP label.
If yes, choose that label, skip the sub-steps, and click **Next>** to proceed to step 4.

3.1 Assuming there is no existing sfGFP label, in **Fluorometry** tab **Add** a new label, name it sfGFP, click **OK.**

3.2 Set **CW-Lamp Energy**: 17000 (depends on excitation wave length)
Set **CW-Lamp Control**: Stabilized Energy
Set **CW-Lamp Filter**: F485 (Excitation filter)
Set **Emission Filter**: F535
Set **Emission Aperture**: Normal (4mm in diameter)
Set **Counter position**: Bottom
Set **Counting time**: 0.2 s
Click **OK**

3.3 Select the label you just create(**sfGFP**) click **OK** then **Next>**

4 Select the wells you are going to measure, (each time when you start a measurement, check and modify the wells you are measuring). Click **Next>**.
If there is no additional information or description you want to add, click **Finish** to start the measurement.

5 A shaking procedure is recommended to be added before measurement.This step tells how to include shaking.

5.1 In **PerkinElmer 2030 Manager** interface:
Select **Protocol editor**(third icon on the second tab row),
Click **Measurement** tab,
On the right there is a column of events, Click **Shake.**

5.2 Set **Shaking duration**: 1.0s

Set **Shaking speed**: Normal
Set **Shaking diameter**: 0.10mm
Set **Shaking type**: Linear
Set **Repeated operation** : No
Click **OK**

5.3 Because we want to shake it before each measurement,
select **Shake** and click the **up-pointing arrow** on the left make sure it's on the top of sfGFP label.

6 Our PURE expression is carried on in 37 °C , set the temperature to 37 °C

6.1 In **PerkinElmer 2030 Manager** interface:
Select **Temperature** tab,
Turn on **Plate heating** option,
Set **target temperature** to 37 °C ,
Click **Apply**

7 Wait until the plate reader reaches the desired temperature, click **Start** icon to start the measurement.
Once a protocol is created it can be used for the same measurement next time, but don't forget to modify the wells you are measuring.