Dual Luciferase Assay

Renilla luciferase reporter activity is expressed relative to integrated Firefly luciferase reporter and is measured in the same protein extracts.
**Standard protocol for dual luciferase assay in S. cerevisiae**

Dual Luciferase Assay System, Promega# E1910

1) For each sample, take 3 colonies from fresh transformation plates and inoculate in appropriate dropout media and grow at 30°C overnight. Note: If internal reporter and test reporter are both integrated in chromosome it is ok to streak them out from glycerol stock. If either of them is on a plasmid it is better to use cells from a fresh transformation.

2) Dilute cells to make starting OD$_{600} = 0.1$, grow them until late log phase (OD$_{600}$ is between 0.8 and 1). Note: Before using a fixed number of cells for this dual luciferase assay, dilute cells in such a way that a series of samples contain 500 to 40,000 cells to check the linearity of the assay.

3) Remove 5 to 10 µl of cells was directly from culture and transfer to 100 µl of 1x passive lysis buffer. After allowing lysis for 10 to 15 seconds, a 10 µl aliquot is used for luminescence measurements. Mix it with 100 µl of LARII in the test tubes by pipetting up and down 2 to 3 times. Do not vortex.

4) Program the luminometer to perform 10-second premeasurement delay, followed by 10-second measurement period for each reporter assay. Note: Use lysis buffer as blank before the measurement of samples.

5) Add 100µl of Stop and Glo reagent and vortex briefly to mix. Replace the sample in the luminometer. Note: To save reagents, the results are still reliable if only use half of reagent volume for each sample. But be sure, the ratio of LARII and Stop & glow reagent is 1:1. For only finding out YES or NO, 5µl LARII and 5µl Stop & glow reagent is enough for one assay.

- The reading can reach to 19,000, 000 for Luminometer (Berthold Detection Systems GmbH, Pforzheim/Germany), however, only a reading below 10,000,000 is in the linear range.