Determination of the ratio of LINK/protein in LINK activated protein

Component	Product size	Storage
	BP-50065	
650-MAGIC	1 mg	-20 °C

Overview

MagicLink ™ technology is the third generation of protein crosslinking technology and has been engineered to allow rapid conjugation of all classes of biomolecules, such as protein, antibody, enzyme, nucleic acid, etc. The proprietary chemistry is based on instant reaction between MAGIC activated biomolecule and LINK activated biomolecule. It is featured as instant conjugation reaction, almost quantitively yield, and most stable linkage on the market.

In some cases, the exact number of LINK per biomolecule is need. 650-MAGIC offers scientists a way to estimate how many LINK moieties were successfully coupled to their molecules or proteins of interest.

In pH 7-9.0, 650-MAGIC instantly reacts with LINK-activated protein to form 650 conjugates. By following the easy protocol below, scientists can calculate the ratio of link/ protein which is the ratio of dye per protein.

Sample Experimental Protocol

1. Reconstitute 650-MAGIC vial with 100 µl DMSO.
2. Add 20X reconstituted 650-MAGIC (MW ~1800 g/mol) to mol of LINK-protein sample in PBS buffer. (Alternatively, use 20 µl of reconstituted 650-MAGIC solution for every 100 µg of LINK activated protein).
3. Mix, incubate at room temperature for 1 hour.
4. Proceed to dye removal step to remove excess 650-MAGIC, and then calculation step.

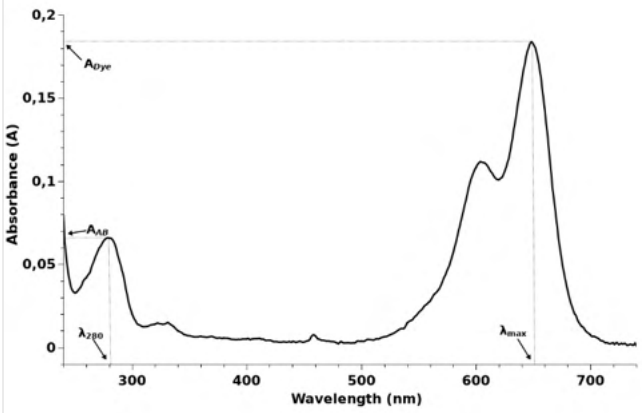
Buffer Exchange and Excess dye Removal

1. Remove bottom closure of Zeba Spin desalting column and loosen, but do not remove, the cap.
2. Place the column in a 1.5-2.0 ml collection tube. Centrifuge at 1,500 × g for 1 minute to remove storage solution.
3. Place a mark on the side of the column where the compacted resin is slanted upward. Place the column in the microcentrifuge with the mark facing outward in all subsequent centrifugation steps.
4. Add 300 µL of 1X PBS on top of the resin bed. Centrifuge at 1,500 × g for 1 minute to remove buffer.
5. Repeat Step 4 two additional times, discarding buffer from the collection tube.
6. Transfer the column to a new collection tube, uncap to add up to 130 µl the 650 dye protein sample to the top of the compact resin bed. (Optional, for sample volumes < 70 µl apply a 15 µl of DI water or buffer to the top of the gel bed after the sample has fully absorbed to maximize sample recovery).
7. Centrifuge at 1,500 × g for 2 minutes to collect the sample. Discard the desalting column after use.

Calculation of Dye to Protein Ratio with a nanodrop UV/Vis Spectrophotometer:

1. Setup nanodrop spectrophotometer and blank it with water.
2. Load 2ul of sample and read A_{650} and A_{280} of the 650 conjugates.

3. Calculate the Dye to Protein Ratio by the formula below.



Dye	Abs Max	Emi. Max.	Ɛ (dye)	CF_{280}
650-MAGIC	648	668	270000	0.03

$$\frac{\boldsymbol{LINK}}{\boldsymbol{Protein}} = \frac{\boldsymbol{A_{650} \times \varepsilon_{protein}}}{(\boldsymbol{A_{280} - 0.03 \times A_{650}}) \times \varepsilon_{\boldsymbol{dye}}}$$

A_{650} : Absorbance at 650 nm of the 650-Protein conjugates,
ε_{bio}: molar extinction coefficient at 280 nm for biomolecules, i.e. antibody or protein
A_{280}: Absorbance at 280 nm of the 650-Protein conjugates,
0.03: correction factor at 280nm accounting for the absorption of the dye at 280 nm
ε_{dye} : molar extinction coefficient of dye 650