Protein Biotinylation Suggestion

Reagents:

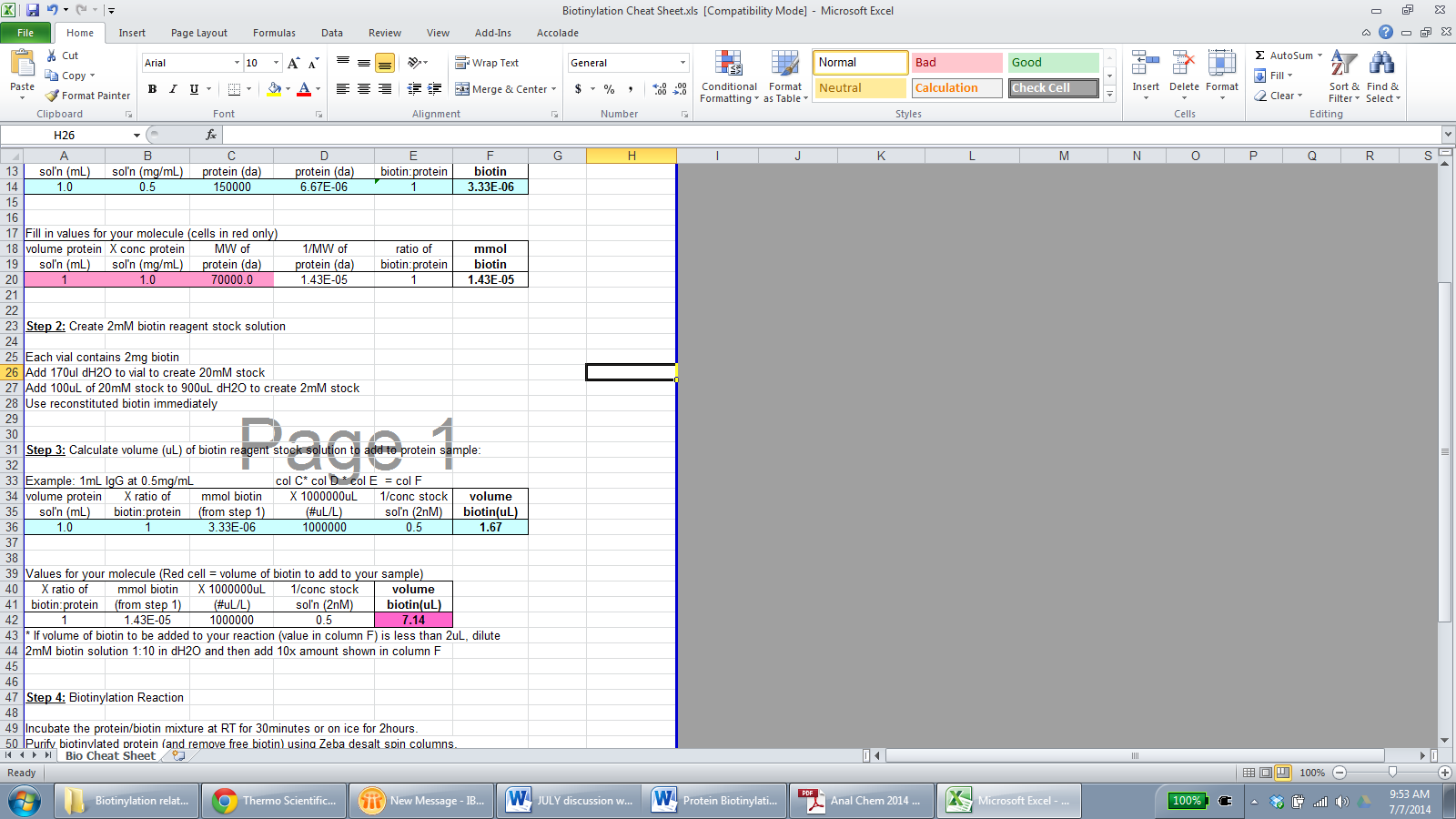
1. **Thermo 21330 NHS-PEO4-Biotin,** 25 mg
2. **Thermo 66382 Slide-A-Lyzer® Dialysis Cassette Kit,** for 0.5-3 ml sample volumes, 10 cassettes, floats and syringes

Optional supply for Buffer Exchange:

1. Pall Microsep UF   OD030C41    24/pk  or    OD030C46    100/pk
2. Vivaspin 500 Ultrafiltration concentrator 30kDa MWCO    Cat.# VS0121   25/pk or VS0122  100/pk
3. Thermo 21455 EZ-Link® NHS-PEO4-Biotinylation Kit [ Not really necessary, you only need the No-Weigh NHS-PEO4-Biotin from the kit. Yet, it includes all buffer and desalting column ]

Procedure:

1. Buffer exchange Protein Sample for biotinylation in Amine Free Buffer. Best to use buffer like PBS. [ No TRIS ]
2. Preforming 1:1 Biotinylation

* Add 170ul of dH20 to one vial of 2mg NHS-PEO4-Biotin to make a 20mM stock
* Dilute the stock 10X with dH20 to make a 2 mM working solution
* Add equal mole of dissolved NHS-PEO4-Biotin to Protein solution to initiate reaction.
* OR use the Biotinylation Cheat Sheet

Just input info in the Pink boxes:

In this example:

1 ml of 1 mg/ml protein at 70,000 Da

Final volume of 2mM working NHS-POE4-Biotin you need will be in this Pink Box. ( 7.14 ul for this example )

1. After the 30 min to 1 hour biotinylation, buffer exchange by dialysis to remove all Free Biotin from the reaction mixture. This step is extremely important. Dialysis overnight is the best way to ensure complete buffer exchange. Using Ultrafiltration filters from Pall or Viva should be fine. Buffer exchange on desalting column is less effective and will lose some of your sample on the column. Nevertheless, it also works.

Remarks:

1. Best biotinylation level is 1:1 or 3:1. Over biotinylation will reduce bioactivity of your protein, unfold the protein upon immobilization, and reduce binding capacity on the Biosensors.
2. For biotinylation of small peptide, one can do 3 – 5 peptide to 1 biotin ratio. This way, all the biotin will be utilized for biotinylation of peptide and dialysis & desalting is not necessary to minimize peptide lost.
3. If possible to construct your protein of interest, utilize AviTag cloning system to introduce site specific biotin to your constructs.

AviTag info

<https://www.avidity.com/t-technology.aspx>

<http://www.genecopoeia.com/tech/avitag-biotinylation-tag/>

Choose the system of choice to clone your Protein of Interest (POI) AviTag system.

Add a 3 – 4 repeat of GGGGS to make the Biotin tag more flexible.

Never over biotinylated your protein. BLI uses Biotinylation for immobilization not for amplification!

1. Never purchase commercially available biotinylated version of your ligand protein. They most likely will be over biotinylated and will not work on ForteBio BLI Technology.
   1. Over biotinylation most likely will block the active site
   2. Over biotinylation will reduce binding capacity on Streptavidin biosensors
   3. Upon immobilization, multiple binding of biotin to SA site on the biosensor will most likely unfold the ligand protein and render its specific activity.