INSTRUCTIONS

ProteinMods

Alkyne quantification kit

Storage: -20° or colder

Background

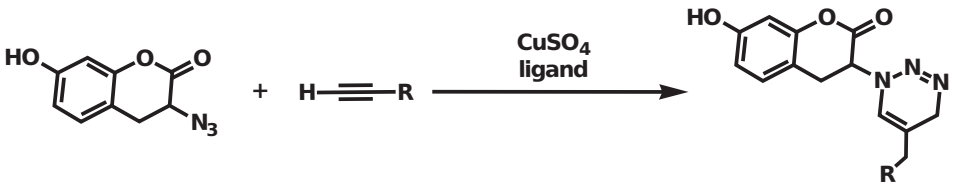
Protein Mods now offers a reagent to quantify alkynes which are accessible for participation in copper-catalyzed, azide-alkyne cycloaddition reactions (CuAAC). Addition of a CuAAC-reactive test sample produces a fluorescent product. By comparing to the defined concentration of the standard (included), the number of reactive alkynes in the test sample can be quantified.

The classic, click-chemistry reaction is the Cu (I) catalyzed azide-alkyne cycloaddition reaction (CuAAC). A component with an accessible azide group can be quickly, efficiently, and covalently attached to a second component with an accessible alkyne group. This reaction is especially well suited for use with biomolecules since it is rapid, largely pH-insensitive, and does not involve components that are hydrolyzed in water (unlike succinimidyl-ester chemistry).

Chemistry

This kit includes two solutions. The quantification reagent is mixed with the test sample to produce fluorescence. The standard solution is already fluorescent. The standard solution is used to confirm the fluorimeter is producing data in the appropriate range and also to establish a standard curve.

The quantification reagent is a modification of a formulation outlined by Presolski, et al (1). It relies on the CuAAC reaction of an alkyne with 3-azido-7-hydroxycoumarin to generate a highly fluorescent product (Sivakumar, K et al) (2). This product has a maximum excitation wavelength of 404 nm and maximum emission wavelength of 477 nm. Additional components include CuSO4, tris(3-hydroxypropyltriazolylmethyl)amine, potassium phosphate, and sodium ascorbate.



The standard solution was generated using the same components as the quantification reagent with the addition of an excess of propargyl alcohol. This results in a solution of 100 uM of CuAAC-activated fluorescent hydroxycoumarin.

Procedure

1. Dilute experimental sample to 250 uM or less using water. Add 20 ul of this sample to 80 ul of quantification reagent.

2. Similarly, add 20 ul of water to another tube containing 80 ul of quantification reagent (negative control).

3. Mix both tubes gently (excessive agitation may introduce too much oxygent and inhibit the reaction).

4. Allow a minimum of 20 minutes at room temperature for reaction to proceed. At this time, thaw standard solution.

5. Prepare 3 standards:
   1. Undiluted standard solution
   2. Twenty-five microliters standard solution plus 75 microliters water
   3. Five microliters standard solution plus 95 microliters water

6. Measure fluorescence of all solutions (excitation at 404 nm, emission at 477 nM).

A proper linear range of measurement is established if the undiluted standard produces approximately 4x and 20x the fluorescence of the diluted standard solutions. If fluorescence of the experimental solution is intermediated to that of the undiluted and diluted standards, then the concentration of CuAAC-reactive alkynes can be extrapolated (undiluted standard contains 100 uM CuAAC-activated fluorescent molecules). If the fluorescence value lies outside these values, concentrate or dilute the original alkyne solution appropriately and repeat the production of the test sample as outlined in step #1. The standard solution and its dilutions are stable for several hours and do not need to be prepared again as long as evaporation can be minimized.

Troubleshooting

Excessive oxygen (from vortexing or other excessive agitation) and oxidizing agents (such as H_2O_2) may oxidize the catalytic Cu(I) to Cu(II) which is non-catalytic.

High salt concentrations (exceeding 1M) may precipitate the non-polar hydroxycoumarin azide creating cloudiness and affecting fluorescent output.

The quantitation reagent is lightly buffered at pH 7.4. Molecules with an isoelectric point near 7.4 may become poorly soluble when added to quantification reagent. If this is an issue, the pH of the quantitation reagent can be adjusted 0.5 pH units in either direction without affecting results.

If dilutions of the standard solution are not linear, adjusting the gain on the fluorimeter may fix the problem.

References

Presolski, S.I., Hong, V.P., and Finn, M.G. Copper-Catalyzed Azide-Alkyne Click Chemistry for Bioconjugation. Curr Protoc Chem Biol. 2011:3(4): 153-162

Sivakumar, K, et al., Xie, F., Cash, B.M., Long, S., Barnhill, H.N., Wang, Q.A. Fluorogenic 1, 3-Dipolar Cycloaddition Reaction of 3-Azidocoumarins and Acetylenes. Org Lett. 2004:(6): 4603-4606