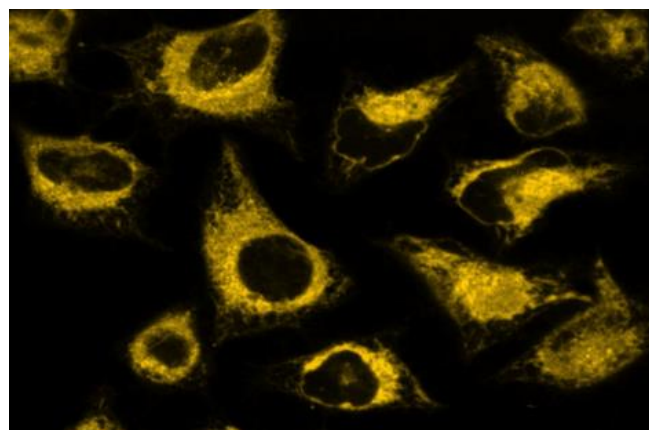


## ReZolve-Alkyne™

### Luminescent Probe For Tagging Molecules

<b>Product Name</b>	ReZolve-Alkyne™
<b>Product Code</b>	1101025
<b>CAS Number</b>	2070860-85-2
<b>Ex/Em</b>	405 nm/ 570 nm
<b>Quantity</b>	0.5 mg
<b>Application</b>	Luminescent Tag



ReZolve-Alkyne™ is a luminescent probe that can be used to tag azide functionalised molecules for detection in cells or tissues. Azide-Alkyne cycloaddition “Click” chemistry is utilized to couple the terminal alkyne to an azide-functionalised molecule of interest, in the presence of catalytic copper. The reactions require simple or no workup or purification of the product. Alone ReZolve-Alkyne™ is a cell permeable tag that has a large stoke shift and excellent resistant to photobleaching which makes it an ideal for fluorescence imaging and compatible with other commercially available fluorescent stains.

#### Specifications

- Suitable for conjugating to azide-functionalised molecules
- Simple “Click” chemistry coupling protocol
- Highly resistant to photobleaching
- Large stoke shift (Ex/Em 405 nm/ 570 nm)
- Compatible with other commercially fluorescent dyes
- Ideal for epi-fluorescence, confocal and multiphoton imaging
- Stable at room temperature

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FLUOROPHORES FOR TARGETED INSIGHTS

## ReZolve-Alkyne™

### Precaution For Use

Please read the entire procedure before performing staining procedure for fixed or live cell imaging and consider the safety data sheet. For laboratory use only. Not fully tested. Not for drug, household, human or veterinary uses.

### Storage Condition

ReZolve-Alkyne™ should be stored at room temperature and protected from light. Product is stable for up to 6 months.

### Considerations

Re-Alkyne is soluble in both  $\text{CH}_2\text{Cl}_2$  and DMF. It is insoluble in  $\text{H}_2\text{O}$ . The choice of solvent system depends on the solubility of the azide used. If an organic soluble azide is used then there is no need to include  $\text{H}_2\text{O}$  in the initial reaction mixture. However,  $\text{H}_2\text{O}$  will still be required in order to solubilise the sodium ascorbate and  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  prior to addition. Below are protocols for coupling of both soluble and non-soluble Azide derivatives or Azide functionalized molecules.

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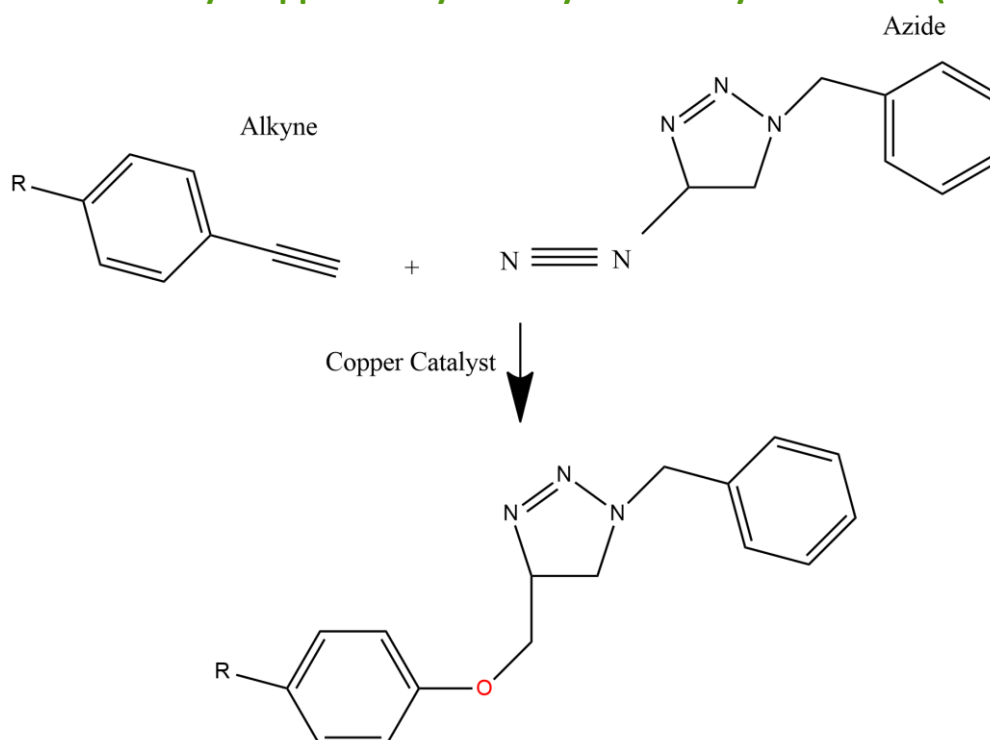
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## ReZolve-Alkyne™

### “Click” Chemistry: Copper catalyzed alkyne-azide cycloaddition (CuAAC)



### Preparation of “Click” Solution

**The click solution** (0.04 M  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ /sodium ascorbate) must be freshly prepared prior to use. Dissolve 16 mg of sodium ascorbate and 8 mg of  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  in 3.2 mL of  $\text{H}_2\text{O}$  to make a 0.04 M solution. Sonicate this mixture at room temperature until a homogenous solution is formed. This should take less than 1 minute. The click solution is now ready for use.

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## FLUOROPHORES FOR TARGETED INSIGHTS

# ReZolve-Alkyne™

### Preparation For Water Soluble Azide

This protocol is for the preparation of water soluble Azide-derivatives or Azide-functionalized molecules of interest.

1. Add 0.5 mg of ReZolve-Alkyne™ ( $8.08 \times 10^{-7}$  mol) with 1:2 molar equivalents of Azide
2. Add 80  $\mu$ L of 4:1 DMF/H<sub>2</sub>O (0.03 M) and 40  $\mu$ L of freshly prepared “Click” solution
3. Mix at room temperature for 16 h or until complete consumption of starting material is observed
4. Concentrate the reaction mixture under a stream of air and suspend the resulting solid in 1 mL of sat. NaHCO<sub>3</sub>
5. Centrifuge the crude material for 5min at 7500 RPM and discard the supernatant
6. Repeat this process using sat. NaHCO<sub>3</sub>, H<sub>2</sub>O and CH<sub>2</sub>Cl<sub>2</sub> successively three times each
7. Dissolve the crude solid in 1 mL of MeOH, filter through a 0.45  $\mu$ m syringe filter and concentrate *in vacuo* to give the desired product

### Preparation For Non-Water Soluble Azide

This protocol is for the preparation of non-water soluble Azide-derivatives or Azide-functionalized molecules of interest.

1. Add 0.5 mg of ReZolve-Alkyne™ ( $8.08 \times 10^{-7}$  mol) with 1:2 molar equivalents of Azide.
2. Add 80  $\mu$ L of 4:1 DMF/H<sub>2</sub>O (0.03 M) and 40  $\mu$ L of freshly prepared “Click” solution.
3. Mix at room temperature for 16 h or until complete consumption of starting material is observed.
4. Dilute the reaction mixture with 1 mL of CH<sub>2</sub>Cl<sub>2</sub> and transfer to a separatory funnel.
5. Wash the organic phase with 1 mL of H<sub>2</sub>O three times, 1 mL of brine, and dry over Na<sub>2</sub>SO<sub>4</sub>, filter.
6. Concentrate the filtrate *in vacuo*.
7. Purify the crude material using column chromatography (e.g. CH<sub>2</sub>Cl<sub>2</sub> to 2% MeOH in CH<sub>2</sub>Cl<sub>2</sub> to 10% MeOH in CH<sub>2</sub>Cl<sub>2</sub>) to give the desired product.

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FLUOROPHORES FOR TARGETED INSIGHTS

## ReZolve-Alkyne™

### Fluorescent Imaging Settings

Please note conjugation of ReZolve-Alkyne™ may alter the photophysical characteristics of the fluorophore. Prior to use the absorbance and fluorescence of your product should be assessed and imaging settings adjusted accordingly.

### Epi-fluorescent Microscopy

ReZolve-Alkyne™ can be excited by UV (~ 365 nm) or blue light (405 nm) sources with emissions collected using a wideband pass filter, or narrowband pass filter within this emission range 550 - 650 nm.

### Confocal or two-photon Microscopy

ReZolve-Alkyne™ can be excited by a 400 nm steady state laser, or 800 - 830 nm using a two-photon pulse laser. Ideally image with a spectral detector set for the emission of ReZolve-Alkyne™, 500-600 nm ( $E_{\max} = 570$  nm). Alternatively detected by using an emission detector suited to the detection of FITC based fluorophores.

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