General Procedure for Photorelease



Together we breakthrough™

This general protocol for the photorelease may be used as a starting point. For optimal result slight tuning of experimental conditions might be required.

- 1. Resuspend the washed resin in 1mL of PBS and transfer to a clear glass vial or quartz cuvette with a tight fitting cap.
- 2. Irradiate the resin suspension with light at 345-375nm with constant agitation for 1 hour. This can be done using hand held long wave UV lamp such as a UVGL-25.1
- 3. Agitate the sample at 37°C for 1 hour after irradiation. Avoid using a stir bar as this can crush some resins.
- 4. Collect the eluant by centrifugation or using an empty spin column.
- Resuspend the resin in 1mL of PBS and agitate for 2-16 hours. For more efficient recovery of enriched protein(s), use a buffer containing 0.1-1% detergent and/or 250mM - 1M NaCl.
- 6. Collect the second elution by centrifugation or using an empty spin column.

Troubleshooting

Problem	Possible Cause	Solution
Poor Photorelease	Light is not sufficiently intense	Use a lamp with a higher intensity.
	Incorrect wavelength of light	Ensure that the lamp is outputting light in the 345-368nm range.
	Insufficient agitation	Ensure that the beads are being properly mixed during photorelease
	Strong non-specific interactions	Consider using a detergent during photorelease or including more wash steps after photorelease

For more efficient photorelease and shorter irradiation time following UV-Lamps can be used:

- http://uvp.com/3uvlamps.html
- http://www.uvsystems.com/store/product.php?productid=16135&cat=250&page=1
- http://www.uvsystems.com/store/product.php?productid=16193&cat=251&page=1

References

- Wang, Z., et al. (2010). Enrichment and Site Mapping of O-Linked N-Acetylglucosamine by a Combination of Chemical/Enzymatic Tagging, Photochemical Cleavage, and Electron Transfer Dissociation Mass Spectrometry. Mol. Cell. Proteom. 9(1): 153-160.
- Olejnik, J., et al. (1995). Photocleavable Biotin Derivatives: A Versatile Approach for the Isolation of Biomolecules. Proc. Natl. Acad. Sci. 92(16): 7590-7594.
- Pandor, M., et al. (2002). Photochemical Control of the Infectivity of Adenoviral Vectors Using a Novel Photocleavable Biotinylation Reagent. Chemistry & Biology, 9(5): 567-573.
- Bai, X., et al. (2003). Photocleavage of a 2-nitrobenzyl Linker Bridging a Fluorophore to the 5' end of DNA. Proc. Natl. Acad. Sci., 100(2): 409-413.
- Zhou, G., et al. (2010). Photocleavable Peptide-Conjugated Magnetic Beads for Protein Kinase Assays by MALDI-TOF MS. Bioconjugate Chem., 21(10): 1917-1924.
- Kim, H., et al. (2009). An Azido-Biotin Reagent for Use in the Isolation of Protein Adducts of Lipid-derived Electrophiles by Streptavidin Catch and Photorelease. Mol. Cell. Proteom., 8(9): 2080-2089.
- Szychowski, J., et al. (2010). Cleavable Biotin Probes for Labeling of Biomolecules via Azide-Alkyne Cycloaddition. J. Am. Chem. Soc., 132(51): 18351-60.