TECH TIP #11

# Light sources and conditions for photoactivation of aryl azide crosslinking and labeling reagents

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## Introduction

Pierce offers a variety of photoreactive crosslinking and biotinylation reagents (see Related Pierce Products). These are useful for many applications including the study of protein:protein interactions, isolating cell surface proteins and preparing labeled probes. With few exceptions, the photoreactive groups in these reagents are aryl azides (Figure 1). When an aryl azide is exposed to UV-light, it forms a nitrene group that can initiate addition reactions with double bonds, insertion into C-H and N-H sites, or subsequent ring expansion to react as a nucleophile with primary amines (Figure 2). The latter reaction path dominates when primary amines are present in the sample.

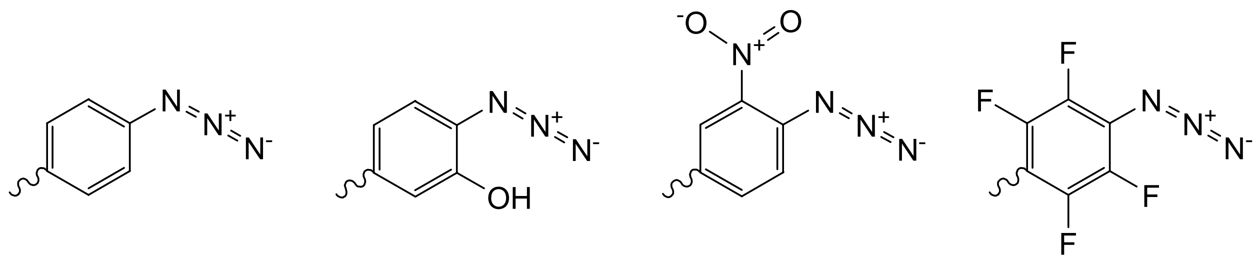


**Figure 1.** Forms of aryl azide reactive groups in photoreactive crosslinking reagents.

A question that often arises with respect to aryl azide linkers is what wavelength and intensity of light is optimal for photoactivation and efficient crosslinking. Pierce researchers have performed only a limited number of experiments to explore these conditions. However, one set of data was generated for the homobifunctional hydroxyphenyl azide crosslinker BASED (Product No. 21564). Experiments with three different lamp sources and several exposure times indicated that activation with long wavelength UV-light (366 nm) for 30 minutes yields the most efficient (complete) crosslinking of the target molecule (in this case, a peptide) and depletion of free crosslinker (Table 1). These data for BASED are probably representative for all the aryl azide reagents, although short wavelength may be better than long wavelength for plain phenyl azides (no hydroxy or nitro group on the phenyl ring). Examination of Table 1 and perusal of the literature (Table 2) indicate that successful photoactivation with these reagents is possible across a wide range of wavelength and time of exposure.

In addition to choosing an appropriate lamp source for photoactivation, consider the following points when preparing conjugation reactions with aryl azide crosslinkers:

- When microcentrifuge tubes are used for the sample, it is most effective to open the cap so that the sample may be exposed directly rather than through the polypropylene sidewall, which shields most of the UV-light. Quartz spectrophotometric cuvettes are an ideal choice for reaction vessels because they allow for optimal exposure of the sample to the UV-light source.
- Samples will become warm or even hot if exposed to intense UV-light for several minutes. Place the sample vial on ice or use some other method to keep the sample cool during UV-light activation.
- Avoid thiol-containing reducing agents (e.g., DTT or 2-mercaptoethanol) in the sample solution during all steps before and during photoactivation. These reagents will reduce the azide functional group to an amine, preventing photoactivation.
- Avoid buffers that contain primary amines (e.g., Tris or glycine) during photoactivation because these will quench the desired reaction. Reaction of the photoactivated aryl azide groups to primary amines dominates if they are present.

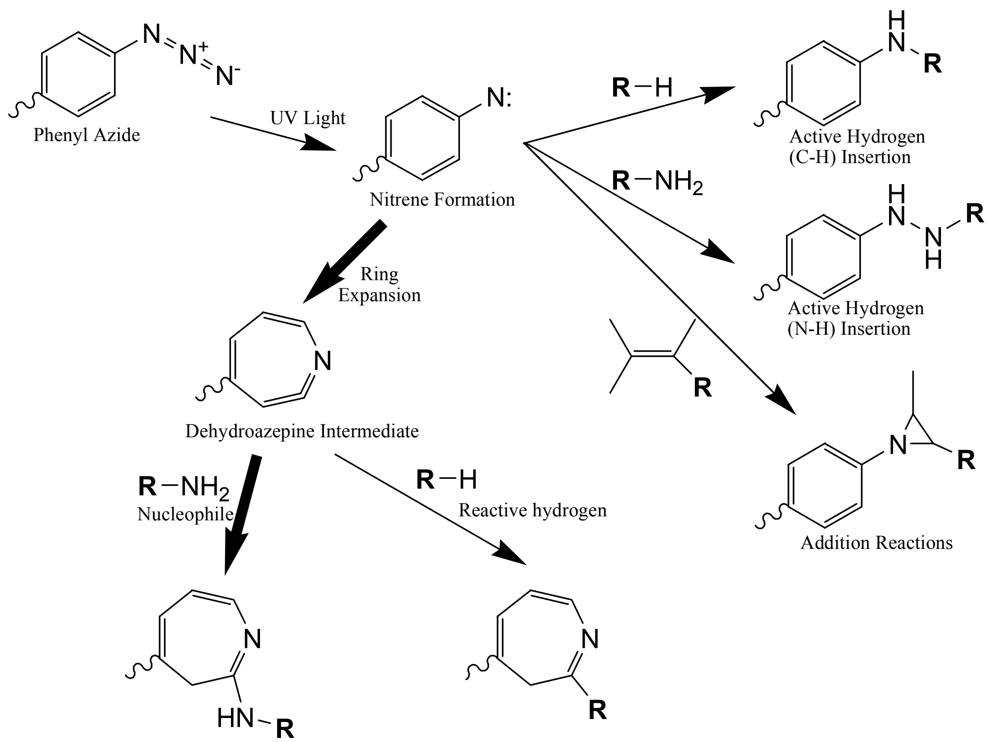


**Figure 2.** Possible reaction pathways of aryl azide crosslinkers.

**Table 1.** Lamp conditions and crosslinking efficiency using BASED (Product No. 21564). Identical reactions with BASED and peptide were prepared and photoactivated by exposure at different wavelengths and lengths of time. Conjugation efficiency was determined by HPLC measurement of the percent peptide and crosslinker depleted relative to the starting material (greater depletion corresponds to more complete crosslinking).

| Sample | Time | % Peptide depleted | % BASED depleted |
|---|---|---|---|
| Long Wave UV-Light | 5 min | 41.46 | 46.79 |
| (366 nm) | 15 min | 47.92 | 77.14 |
| | 30 min | 61.46 | 94.33 |
| Short Wave UV-Light | 5 min | 11.26 | 9.61 |
| (254 nm) | 15 min | —— | —— |
| | 30 min | 14.46 | 24.82 |
| 550 Watt Light | 10 sec | 27.87 | 31.51 |
| (Broad spectrum visible) | 30 sec | —— | 30.52 |
| | 60 sec | 42.43 | 58.54 |
| 550 Watt Light | 6 flashes, each 1 sec | 3.82 | 18.38 |
| (Broad spectrum visible) | 12 flashes, each 1 sec | 19.50 | 29.95 |
| | 18 flashes, each 1 sec | 12.26 | 32.23 |
| Combination | 15 min Long UV-Light | 35.23 | 74.60 |
| | Plus 12 flashes | 37.76 | 76.54 |

**Table 2.** Lamp sources and conditions used for photoactivation of aryl azide crosslinkers. The information in this table was compiled from older (pre-2000) literature references and is provided only to exemplify the types of conditions used by researchers.

| Lamp | Distance from sample | Wavelength | Time |
|---|---|---|---|
| Rayonet Photochemical Reactor (UltraViolet Products, Inc.) | 2 cm | 300 nm | ?* |
| Model UVGL-15 Mineralight | ? | 254-360 nm | 10 min |
| ? | 3.4 cm | 366 nm | 5 min |
| Fotodyne UV Transilluminator | 5 cm | ? | 2 min |
| 750 W mercury lamp (Scaeffel Instrument Co.) | 20 cm | 320 nm | ? |
| Black Ray model XX-15C, 0.41 A (Black Ray UV Products, Inc.) | 18 cm<br>2.5 cm<br>10 cm | ?<br>?<br>? | 75 min<br>2-30 min<br>5 min |
| Edmund Scientific No. 60889 (9 W, 12 in. UV tube) | 2 mm | >300 nm | 120 min |
| Mineralight UVS-11 | 1 cm | ? | 5 min |
| Black Ray Model B-100A | 10 cm | >300 nm | 5 min |
| ACME LITE model 228A electronic flash (cover removed) | 1 cm | ? | 20 flashes |
| Transilluminator UV light box (UltraViolet Products, Inc.) | 3.5 cm | 302 nm | 5 min |
| Sun Thyristor Flash unit (1/4 power) | 2 cm | ? | 20 flashes |
| 500 W Xenon lamp | 9 cm | ? | 2-10 min |
| Zeiss 100 W mercury lamp | 15 cm | ? | ? |
| 250 W sunlamp (General Electric) | 15 cm | ? | 15 min |
| 15 W UV lamp | 1 cm | 365 nm | 10 min |
| Kako Auto 201 electronic flash (plastic cover removed) | 3-5 cm | ? | 5 flashes |
| 50 W HBO mercury lamp (OSRAM, Berlin) | ? | ? | 30 sec |
| UVS-11 Mineralight | 5 cm | ? | 3 min |
| 100 W HBO-101 xenon lamp (Narva, Berlin) | 1 cm | ? | 70 sec |
| UVS-52 | 15 cm | ? | 3 min |
| Rayonet UV Light Reactor (Southern N.E. Ultraviolet Co.) | 10 cm | 370 nm | 5 min |
| 500 W xenon lamp | 9 cm | ? | 10 sec |
| Chromato-Vue C3 viewing box (Ultraviolet Products, Inc.) | 10 cm | 254 and 365 nm | 20 min |
| Model AH-6 1 kW high pressure Hg lamp (Advanced Radiation) | 10 cm | ? | 5 sec |
| Universal UV lamp (Camag Muttenz, Switzerland) | 1 cm | 254 nm | 2 min |
| UV SL-25 4 W Mineralight (Ultraviolet Products, Inc.) | 1 cm | 254 cm | 10 min |

## Related Pierce Products (Photoreactive reagents offered by Pierce)

| | | Reactive Groups | | Spacer Arm | |
|---|---|---|---|---|---|
| **Product Name** | **Product No.** | **Photoreactive** | **Other Group(s)** | **Length (Å)** | **Cleavable?** |
| **ABH** | **21510** | Phenyl azide | Hydrazide | 11.9 | No |
| **ANB-NOS** | **21451** | Nitrophenyl azide | NHS | 7.7 | No |
| **APDP** | **27720** | Hydroxyphenyl azide | Pyridyldisulfide | 21.0 | Yes |
| **APG** | **20108** | Phenyl azide | Phenylglyoxal | 9.3 | No |
| **ASBA** | **21512** | Hydroxyphenyl azide | Amine | 16.3 | No |
| **BASED** | **21564** | Hydroxyphenyl azide | Hydroxyphenyl azide | 34.7 | Yes |
| **Mts-Atf-Biotin**<br>**Mts-Atf-LC-Biotin** | **33093**<br>**33083** | Tetrafluorophenal azide | Methanethiosulfonate and Biotin | 11-31<br>21-35 | Yes |
| **NHS-ASA** | **27714** | Hydroxyphenyl azide | NHS | 8.0 | No |
| **SADP** | **21533** | Phenyl azide | NHS | 13.9 | Yes |
| **SANPAH** | **22600** | Nitrophenyl azide | NHS | 18.2 | No |
| **SPB** | **23019** | Psoralen | NHS | 8.6-9.5 | No |
| **Sulfo-HSAB** | **21563** | Phenyl azide | Sulfo-NHS | 9.0 | No |
| **Sulfo-NHS-LC-ASA** | **27735** | Hydroxyphenyl azide | Sulfo-NHS | 18.0 | No |
| **Sulfo-SADP** | **21553** | Phenyl azide | Sulfo-NHS | 13.9 | Yes |
| **Sulfo-SAED** | **33030** | Azido-methylcoumarin | Sulfo-NHS | 23.6 | Yes |
| **Sulfo-SAND** | **21549** | Nitrophenyl azide | Sulfo-NHS | 18.5 | Yes |
| **Sulfo-SASD** | **27716** | Hydroxyphenyl azide | Sulfo-NHS | 18.9 | Yes |
| **Sulfo-SFAD** | **27719** | Perfluoroaryl azide | Sulfo-NHS | 14.6 | Yes |
| **Sulfo-SANPAH** | **22589** | Nitrophenyl azide | Sulfo-NHS | 18.2 | No |
| **Sulfo-SBED** | **33033** | Phenyl azide | Sulfo-NHS/Biotin | 14-25 | Yes |
| **Psoralen-PEO-Biotin** | **29986** | Psoralen | Biotin | 36.9 | Yes |
| **Photoactivatable Biotin** | **29987** | Nitrophenyl azide | Biotin | 30.0 | No |
| **Biotin-LC-ASA** | **29982** | Hydroxyphenyl azide | Biotin | 29.9 | No |
| **TFPA-PEO$_3$-Biotin** | **21303** | Tetrafluorophenal azide | Biotin | 33.4 | No |

### References

1. Hermanson, G. T. (1996). Bioconjugate Techniques, Academic Press, Inc., p. 163, 252-255.


**Note:** This book is available from Pierce as Product No. 20002.