Application Note · UVP Crosslinker



Challenge

Small-scale high-intensity, highuniformity ultraviolet light

Solution

Integrating UVP CL-3000 into ultraviolet applications

History of Excellence: Ultraviolet-based Applications with the UVP Crosslinker

Introduction

Ultraviolet light is part of the electromagnetic spectrum that spans from 100-400 nm. The ultraviolet spectrum is broken up into three categories, UVA, UVB and UVC (Table 1). On Earth, UVC is blocked by molecular oxygen in the atmosphere, while UVA and UVB readily travel to the Earth's surface. Anyone who has experienced a sunburn, can testify to the dangerous power of ultraviolet light. While ultraviolet can be harmful, it has helped improve human health from direct applications to sanitation to our fundamental understanding of how UV affects our biology.

UV Common Name	Wavelength (nm)	UVP Crosslinker, CL-3000 Wavelength (nm)
UVA	315-400	365
UVB	280-315	302
UVC	200-280	254
Vacuum UV	100-200	NA*

*Analytik Jena does not manufacture Vacuum UV products

Table 1. Ultraviolet Wavelengths



For over 120 years, ultraviolet light has been used in applications for preventing the growth of microorganisms on surfaces, destruction of microorganisms in the air and sterilization of liquids. For example, since 1877, humans have used ultraviolet light for food preservation/sterilization¹; In the 1930s, ultraviolet light started being used in operating rooms to sterilize the air²; and on and off since the early 1900s, ultraviolet light has been used to sterilize drinking water³. The same properties that give ultraviolet light its effectiveness in the aforementioned applications, make it dangerous to biological organisms.

At the molecular level, upon UV exposure, adjacent pyrimidines in a nucleic acid strand can undergo dimerization, which occurs predominately between thymine bases^{4–6}. For nearly 100 years, researchers have taken advantage of this property of ultraviolet light as a tool in studying genetic mutations. In fact, had earlier attempts to harness the mutagenic properties of UV light been successful⁷, UV-induced, not X-ray induced⁸ mutations would be tied to Herman Muller's landmark 1928 fly experiments, which later earned him the 1946 Nobel Prize–after all, ultraviolet light became a popular strategy in his lab later in his career^{9–16}. But alas, researchers were late to identify the optimal wavelength of ultraviolet light that bestowed the mutagenic properties (i.e. 254 nm or UVC).

Separately and about a decade before Muller's landmark work, researchers observed that ultraviolet light produced coagulates in titrated protein solutions^{17,18}. Protein researchers would later understand the underlying mechanism that produced this coagulation was the modification of tryptophan residues and disruption of disulfide bridges¹⁹. Collectively, these discoveries paved the way for ultraviolet-based applications in molecular and cellular biology, and beyond.

At Analytik Jena we have been manufacturing the UVP Crosslinker since 1993 using UVA, UVB, and UVC light to cover an array of applications summarized in Figure 1 (Visit our Crosslinker <u>webpage</u> for a comprehensive list of publications using our crosslinkers). These applications include:

- Mutation biology (UV-induced mutations, survival assays, stress assays)
- Membrane crosslinking (Northern/Southern blotting, EMSA)
- Bimolecular crosslinking (CLIP-seq, iCLIP-seq, PAR-CLIP-seq, pBpa, sulfo-SDA, psoralen)
- Microorganisms inactivation (e.g. bacteria, fungus, virus)
- Polymer curing (e.g. hydrogels, methyacrylate resins)
- Photostability verification



Figure 1. Applications Summary of UVP Crosslinkers from Pub Med Central Using Textspresso. We mined Pub Med Central using Textspresso²⁰ to identify unique hits for UVP Crosslinkers. We identified 212 unique hits, which are classified to broad categories for simplicity.

These applications are reliant on high-intensity, reproducible UV doses. Our most recent model, the UVP Crosslinker CL-3000 (Figure 2A), is designed with a built-in radiometer calibrated to a NIST traceable standard—this ensures consistent doses irrespective of space and time. In addition, the CL-3000 can produce a cumulative dose of up to 10 J/ cm². By having reflective housing and the UV source in close proximity to the sample, we are able to achieve highly uniform illumination (Figure 2B and 2C). The coefficient of variation across the entire illumination surface is 17.3%, while the center of the illumination surface is 6.0%. As with all our instruments, safety of the end user is critical, and all our Crosslinkers have a safety-interlock to prevent accidental UV exposure.

Operating the Analytik Jena UVP Crosslinker CL-3000

The Analytik Jena UVP Crosslinker CL-3000 is capable of user inputs in the form of time or fluence/dose in mJ/cm². Given that the age of the bulbs, and environmental variables can affect UV output, we recommend users defer to entering fluence rather than time. In the scientific literature, users will discover that both are used. If fluence is reported, users can simply convert to mJ/cm², which is the unit of measure we use for inputs on our instrument. An example conversion is displayed in Table 2, under **Fluence**.



plot summary of radiometer readings. Total CV: Mean=104.9, SD=18.16; Center CV: Mean=117.9, SD=7.1. We recommend users place samples in the center of the illumination surface. Samples placed in the center will receive, at a minimum, the input energy dose. If users want to place samples at the front-most and back-most positions, we recommend purchasing a UVP 254 nm radiometer (P/N 97-0015-02) and sensor (97-0016-01) to calibrate dose.

If **Fluence rate** is reported, typically in μ W/cm², this value will be accompanied by an exposure time. Users will need to convert **Fluence Rate** into mW/cm² (See example conversion in Table 2, under **Fluence Rate**). In addition, users will need to convert exposure time into seconds (e.g. 10 minutes = 600 seconds). See example scenario and calculation below.

Example Scenario and Calculation

A researcher exposes influenza A virus to 1,000 uW/cm² for 10 minutes. Convert the reported UV dose into mJ/cm².

$1 \text{ mW/cm}^2 \times 600 \text{ sec} = 600 \text{ (mJ/cm}^2)$				
Fluence				
µJ/cm²	mJ/cm²	J/cm ²	J/m ²	
1,000,000	1,000	1	10,000	
Fluence Rate				
µW/cm²	mW/cm²	W/cm ²	W/m²	
1,000,000	1,000	1	10,000	

Fluence Rate $(mW/cm^2) \times Time (sec) = Fluence/Dose (mJ/cm^2)$ Equation 1.

Table 2. Important Conversions for Ultraviolet Light Measurements

*Values in the literature are most commonly reported as fluence in mJ/cm² or J/m². In some cases users report the fluence rate (e.g. µW/cm²), in which case user should use Equation 1 to determine fluence (or dose).

Placement of Samples

We recommend users place samples in the center of the illumination surface where the CV=6% (Figure 2B, 2C). Samples placed in the center will receive at a minimum, the input energy dose. Samples placed at the front-most and back-most positions will receive less than the input dose, due to edge effects. If users want to calibrate the UV dose at the edges, we recommend purchasing a UVP 254 nm radiometer (P/N 97-0015-02) and sensor (97-0016-01) to determine the lowest UV dose on the illumination surface of their instrument. For example, Figure 2B displays the front-most and right-most position as the lowest dose (78.18 mJ/cm²) when we input 100 mJ/cm². Therefore, the user may consider inputting a higher dose (e.g. 125 mJ/cm²), to account for edge effects.

At Analytik Jena, we are excited to continue being part of the innovations that take place with UV-based technologies. For more information about the UVP Crosslinker CL-3000, see the Technical Specifications and Ordering Information below. You can also contact our Customer Service or Technical Support Department at 909-946-3197, Monday-Friday 7am-4pm PT.

Technical Data

Technical Specifications	CL-3000
Wavelength	254 nm, 302 nm, 365 nm
Bulbs	6 x 8 Watt
Energy	0000.1 - 9999.9 mJ/cm² (0 - 10 J/cm²)
Time	000:01 - 999:59 mmm:ss (>300J/cm ²)
Temperature	15°C - 35°C
Humidity	70% Non-Condensing
Altitude	up to 3,000M (9,842 ft)
Sound Level	≤ 50 dba
Housing Surface Temp	≤ 30°C
Startup Time	< 1 sec
External Dim (L x W x H)	41cm x 40cm x 26.5cm
Internal Dim (L x W x H)	35cm x 27cm x 16cm
Weight	6.8Кg: 15 lb
Operating Power	100 - 115VAC & 230VAC 50/60Hz
Certifications	CE, RoHS (CSA In Process)

Part Numbers

Part Number		Description
115V	230V	
849-95-0615-01	849-95-0615-02	UVP Crosslinker (CL-3000), 254 nm
849-95-0615-03	849-95-0615-04	UVP Crosslinker (CL-3000M), 302 nm
849-95-0615-05	849-95-0615-06	UVP Crosslinker (CL-3000L), 365 nm
34-0007-01	849-057-007	Replacement UV tube, 8 watt, 254 nm
34-0042-01	-	Replacement UV tube, 8 watt, 302 nm
34-0006-01	-	Replacement UV tube, 8 watt, 365 nm

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