INSTRUCTIONS

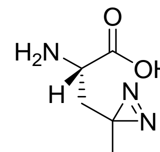
Thermo SCIENTIFIC

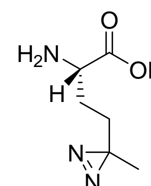
L-Photo-Leucine
L-Photo-Methionine

22610 22615

1984.2

Number	Description
22610	**L-Photo-Leucine** (L-2-amino-4,4-azi-pentanoic acid), 100 mg Molecular Weight: 143.15 Formula: $C_5H_9N_3O_2$
22615	**L-Photo-Methionine** (L-2-amino-5,5-azi-hexanoic acid), 100 mg Molecular Weight: 157.17 Formula: $C_6H_{11}N_3O_2$





Storage: Upon receipt store at 4°C in dark. Product shipped at ambient temperature.

Introduction

L-Photo-Leucine and L-Photo-Methionine are amino acid derivatives that contain diazirine rings for ultraviolet (UV) photo-crosslinking of proteins. These photo-active amino acids substitute for their respective natural amino acids and are incorporated directly into proteins using the endogenous mammalian translation machinery. The overall incorporation rate is 10-20%, depending on amino acid frequency, sequence length, protein abundance and protein turnover. Photoactivation of the diazirine rings creates reactive carbene intermediates that irreversibly crosslink proteins with a zero-length bond. Crosslinking occurs within protein-protein interaction domains and results in > 90% recovery of crosslinked proteins after cell lysis. Using L-Photo-Leucine and L-Photo-Methionine together maximizes the probability of crosslinking protein complexes. The resulting complexes have an increased molecular weight that is apparent by SDS-PAGE and Western blot. Other methods for detecting the crosslinked product include size exclusion chromatography, sucrose density gradient sedimentation and mass spectrometry.

Important Product Information

- L-Photo-Leucine and L-Photo-Methionine are stable under typical laboratory lighting conditions; however, avoid prolonged exposure to light.
- Because both leucine and methionine are essential amino acids present in cell culture media, these photo-active derivatives must be used in combination with DMEM minus methionine and leucine (Dulbecco's Modified Eagle's Limiting Medium (minus L-Leucine and L-Methionine [DMEM-LM], Product No. 30030) and PBS-dialyzed serum (Product No. 89986). Incubating cells with DMEM-LM media decreases cell growth from the lack of essential amino acids. Cell viability is not adversely affected by adding photo-amino acids when used at the indicated concentrations.
- For best results, use L-Photo-Leucine and L-Photo-Methionine together to maximize the potential for crosslinking protein complexes. If using only one of the photo-amino acids, supplement DMEM-LM media with 105 mg/L of tissue-culture grade L-leucine or 30 mg/L tissue-culture grade L-methionine, depending on which amino acid is deficient.
- L-Photo-Leucine and L-Photo-Methionine are soluble in aqueous solutions up to 10 mg/ml and 6 mg/ml, respectively.

Photoactivation Information

- Use a UV lamp that irradiates from 320 to 370 nm (see **Note** below) for photoactivation. High wattage lamps are more effective and require shorter exposure times than low wattage lamps. Suggestions for lamps include the Stratalinker 2400 (5 × 15 watt bulbs, emission at 365 nm), mercury vapor lamps (200 watt, 300-360 nm), and Spectroline or UVP hand-held lamps (8 watt, emission at 365 nm). Using lower-wattage hand-held lamps, such as 6 watt lamps, will result in lower crosslinking efficiencies.

  Note: The optimal wavelength for photoactivation is 345 nm. Do not use UV lamps that emit light at 254 nm as this wavelength causes proteins and DNA to photodestruct. Filters that remove light at wavelengths below 300 nm are necessary for mercury vapor lamps.

- Perform UV irradiation in a shallow, uncovered reaction vessel/plate for maximum efficiency. Irradiation efficiency decreases logarithmically with increased distance from the light source. Position a UV lamp 3-5 cm from cells for 15 watt lamps. For lower-powered, hand-held lamps, use a distance of 1 cm without filter, if possible. For lamps > 150 watts, use a distance of 20 cm with a 300 nm filter. Perform photoactivation by placing the lamp above the open reaction vessel to avoid impeding irradiation by the vessel. Samples may need to be rotated for even UV irradiation. Use a total UV irradiation time less than 15 minutes for *in vivo* crosslinking of live cells.

Additional Materials Required

- Dulbecco's Modified Eagle's Limiting Medium minus L-Leucine and L-Methionine (DMEM-LM) (Product No. 30030)
- Rapidly growing cell line plated at 60-70% confluency in tissue culture dishes in normal DMEM media
- Phosphate-buffered saline (PBS): 0.1 M phosphate, 0.15 M sodium chloride; pH 7.2 (Product No. 28372)
- Dialyzed serum (e.g., Dialyzed FBS, Product No. 89986)
- UV lamp with 365 nm bulb(s)

Procedure for *In Vivo* Protein Crosslinking

The following protocol is an example application for this product. Specific applications will require optimization.

A. Photo-reactive amino acid half-life determination

1. Dissolve photo-amino acid in PBS at 1 mg/ml and transfer to a reaction vessel. Remove a portion to determine initial absorbance.
2. Turn on UV lamp 5 minutes before irradiating samples.
3. Position the UV lamp above the vessel and UV irradiate sample for 30 minutes, removing a portion of the sample every 2-5 minutes.
4. Measure the absorbance of each sample portion in a quartz cuvette or UV microplate using a spectrophotometer at 345 nm and blanked with PBS.
5. Determine time needed for half of the sample to be photo-activated as determined by a 50% decrease in absorbance at 345 nm in comparison to starting material (Figure 1).
6. Use five times the half-life for total irradiation time for experiment (e.g., 1 minute half life = 5 minutes total experimental irradiation).

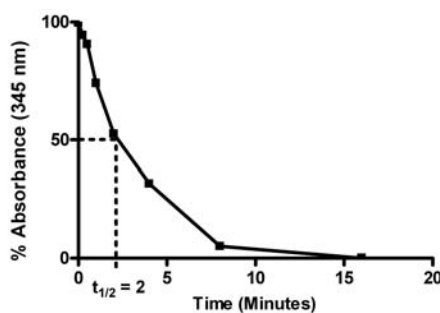


Figure 1. Photo-amino acid half-life using the Stratalinker 2400 with 15W 365 nm bulbs at 3 cm.

B. Photo-Amino Acid Treatment

1. Add 50 ml of PBS-dialyzed serum to 450 ml of DMEM-LM for a final concentration of 10% serum. If necessary, add antibiotics (penicillin/streptomycin) and L-glutamine to the media.

   Note: DMEM-LM supplemented with PBS-dialyzed serum is stable for up to 4 weeks at 4°C.

2. Add photo-methionine and photo-leucine to supplemented DMEM-LM to a final concentration of 2 mM and 4 mM, respectively (4 mM L-Photo-Leucine = 28.6 mg/50 ml; 2 mM L-Photo-Methionine = 15.7 mg/50 ml).

   Note: L-Photo-Methionine is less soluble than L-Photo-Leucine and may require grinding of large crystals. To fully dissolve, warm media to 37°C before adding the photo amino acids or vigorously mix (vortex) after adding.

   Note: For best results, dissolve the photo amino acids just before use. Long-term stability of L-Photo-Leucine and L-Photo-Methioine in solution is unknown.

3. Remove media from cells grown to 60-70% confluency and wash twice with PBS.
4. Add DMEM-LM containing 2 mM photo-methionine and 4 mM photo-leucine to cells and incubate in a tissue-culture incubator for 24 hours.

C. UV Crosslinking of Sample

1. Remove media containing photo-amino acids from cells and wash twice with PBS.
2. Add sufficient PBS to completely cover cells to prevent drying during UV irradiation.
3. Position cells 1-5 cm from UV bulbs and irradiate for five times the half-life ($t_{1/2}$) as established in Section A. Rotate dish during irradiation for even activation. For best results, irradiate for ≤ 15 minutes.
4. Harvest cells for lysis and analyze crosslinked proteins by Western blot or other method.

Troubleshooting

Problem	Possible Cause	Solution
No crosslinking as determined by Western blot	Did not use limiting media and dialyzed serum	Use DMEM-LM limiting media and PBS-dialyzed serum
	Protein is not in a complex	Blot for a model abundant protein such as hsp90 as a positive control
	Protein binding sites do not contain methionine or leucine	Use a lysine- or cysteine-reactive chemical crosslinker
	Protein is of low abundance or poor antibody-epitope recognition	Overexpress the epitope-tagged protein
	UV light source is too weak	Increase UV irradiation time
		Decrease distance to UV bulbs
		Use multiple UV bulbs with > 8 W output and remove UV filters
	Protein complex is too large after crosslinking for SDS-PAGE resolution	Analyze crosslinked proteins by size exclusion chromatography or sucrose density gradient
	Photo amino acids did not completely dissolve in the media	Break up large particles before adding the photo amino acids to media
		Add compounds to smaller volume of media and vortex
		Warm media to 37°C before adding the photo amino acids

Troubleshooting continued

Problem	Possible Cause	Solution
Crosslinking observed in controls without photo-amino acid added	Proteins crosslinked to DNA using 254 nm bulbs	Use only 320-370 nm UV wavelengths
	Tyrosine residues in binding domains crosslinked with UV treatment alone	Denature protein complexes thoroughly by boiling in reducing sample buffer > 5 minutes
Poor crosslinking yields	Cells dried during UV irradiation	Completely cover cells with PBS before irradiation
		Place cells on ice during incubation when using lamps that generate excessive heat
Cell toxicity or cell morphology changed	Cell line-specific toxicity or photo-amino acid concentration is too high	Adapt cell line to DMEM media
		Reduce amount of photo-amino acid in media < 4 mM
		Increase cell confluency to at least 70% before photo-amino acid incubation
		Decrease incubation time with photo-amino acids
		Use rapidly growing cell line

Related Thermo Scientific Products

30030 **Dulbecco's Modified Eagle's Limiting Medium (without L-Leucine and L-Methionine)** (DMEM-LM), 500 ml

89986 **Dialyzed FBS,** 50 ml

28372 **BupH™ Phosphate Buffered Saline Packs,** 40 packs

78501 **M-PER® Mammalian Protein Extraction Reagent**, 250 ml

89901 **RIPA Lysis and Extraction Buffer,** 250 ml

78410 **Halt™ Protease Inhibitor Cocktail Kit**

23227 **Pierce® BCA Protein Assay Kit**

34076 **SuperSignal® West Dura Extended Duration Substrate**, 200 ml

Reference

1. Suchanek, M., *et al.* (2005). Photo-leucine and photo-methionine allow identification of protein-protein interactions. *Nat Methods* **2(4)**: 261-267.

SuperSignal® Technology is protected by U.S. Patent #6,432,662.
Photo-Leuceine and Photo-Methionine are for research use only and protected by Max-Planck Institute for Research: EP 1 700 849 A1

This product ("Product") is warranted to operate or perform substantially in conformance with published Product specifications in effect at the time of sale, as set forth in the Product documentation, specifications and/or accompanying package inserts ("Documentation") and to be free from defects in material and workmanship. Unless otherwise expressly authorized in writing, Products are supplied for research use only. No claim of suitability for use in applications regulated by FDA is made. The warranty provided herein is valid only when used by properly trained individuals. Unless otherwise stated in the Documentation, this warranty is limited to one year from date of shipment when the Product is subjected to normal, proper and intended usage. This warranty does not extend to anyone other than the original purchaser of the Product ("Buyer").

No other warranties, express or implied, are granted, including without limitation, implied warranties of merchantability, fitness for any particular purpose, or non infringement. Buyer's exclusive remedy for non-conforming Products during the warranty period is limited to replacement of or refund for the non-conforming Product(s).

There is no obligation to replace Products as the result of (i) accident, disaster or event of force majeure, (ii) misuse, fault or negligence of or by Buyer, (iii) use of the Products in a manner for which they were not designed, or (iv) improper storage and handling of the Products.

Current versions of product instructions are available at www.thermo.com/pierce. For a faxed copy, call 800-874-3723 or contact your local distributor.

© 2008 Thermo Fisher Scientific Inc. All rights reserved. Unless otherwise indicated, all trademarks are property of Thermo Fisher Scientific Inc. and its subsidiaries. Printed in the USA.

Pierce Biotechnology 3747 N. Meridian Road PO Box 117 Rockford, IL 61105 USA (815) 968-0747 (815) 968-7316 fax www.thermo.com/pierce