Protein Analysis, Modification and Interaction

PN

ΡN

NEW

NEW

Label transfer reagents

Sulfo-SMCC, Thermo Scientific Pierce

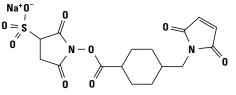
SCIENTIFIC

Provides stable maleimide activated proteins.

- Useful for immunogen formation
- Water soluble
- Cyclohexane ring in spacer stabilises maleimide group
- Reactive groups: sulfo-NHS ester and maleimide
- Reactive toward: amino and sulfhydryl groups

Catalogue No	Description	Quantity
PN22322	Sulfo-SMCC (Sulfosuccinimidyl 4-[N-maleimidomethyl]-cyclohexane-1-carboxylate)	50mg
PN22122	Sulfo-SMCC	1g





Sulfo-SMCC M.W. 436.37 Spacer Arm 8.3 Å

Photo-reactive amino acid, Thermo Scientific Pierce

Thermo

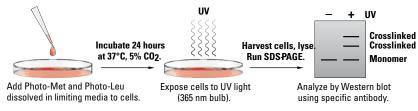
Perform in vivo crosslinking of native protein interactions.

- In vivo crosslinking find interacting proteins in the native cellular environment
- Increased specificity compared to traditional methods crosslink interacting proteins correctly positioned at their interfaces within protein interaction domains
- Efficient recovery 90% protein recovery in cell lysates after crosslinking
- Compatible crosslink proteins expressed in a wide variety of cell lines, including HeLa, 293T, COS7, U2OS, A549, A431, HepG2, NIH 3T3 and C6

Thermo Scientific L-Photo-Leucine and L-Photo-Methionine are analogues of L-Leucine and L-Methionine amino acids that have activatable diazirine side chains capable of chemical crosslinking to adjacent molecules when exposed to ultraviolet light.

When used in combination with specially formulated limiting cell media that is devoid of leucine and methionine, the photo-activatable derivatives are treated like naturally occurring amino acids by the protein synthesis machinery. As a result, they can be substituted for leucine or methionine in the primary sequence of proteins. When exposed to UV light the diazirine rings become reactive intermediates that form covalent bonds with nearby protein side chains and backbones. Naturally associating binding partners are then instantly trapped. Crosslinked protein complexes are detected by decreased mobility on SDS-PAGE followed by Western blot detection (see Figure), size-exclusion chromatography, sucrose density-gradient sedimentation or mass spectrometry.

Catalogue No	Description	Quantity
PN22610	L-Photo-Leucine (L-2-amino-4,4'-azi-pentanoic acid)	100mg
PN22615	L-Photo-Methionine (L-2-amino-5,5'-azi-hexanoic acid)	100mg
PN30030	Dulbecco's Modified Eagle's Limiting Medium (DMEM-LM) Sterile filtered (-)L-leucine, (-)L-methionine with 4.5g/L glucose, 4.0mM L-glutamine, sodium pyruvate and phenol red	500mL



Protocol summary for protein interaction experiments with Thermo Scientific Pierce photo-activated amino acids.

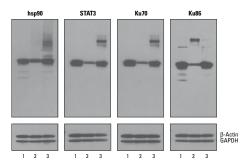
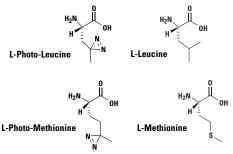


Photo-reactive amino acid crosslinking and formaldehyde crosslinking are complementary techniques for protein interaction analysis. HeLa cells were mock-treated (Lane 1), treated with 1% formaldehyde for 10 minutes (Lane 2), or treated with Photo-Methionine and Photo-Leucine followed by UV treatment (Lane 3). Cells were lysed and 10µg of each was analysed by SDS-PAGE and Western blotting with antibodies against hsp90, STAT3, Ku70 and Ku86. β -actin and GAPDH were blotted as loading controls.



Structures of L-Photo-Leucine and L-Photo-Methionine and their natural analogues.