Contents of Hyas Kit

Iridium Photocatalyst (Yellow Solid) (Red cap – Catalog # IR02 Acid for Hyas), 25 µMoles

Diazirine-PEG3-biotin (Purple cap – Catalog # DZ01), 1 x 5 mg

Hyas conjugation procedure

A dark 8 mL vial was charged with Ir-acid (hyas) (1 equiv., 25 µMoles), Pybop (19.5 mg, 1.5 equiv), and a stir bar. The vial was sealed and evacuated under vacuum and backfilled with nitrogen (x3) and subsequently left under a nitrogen atmosphere. To this was added 1 mL dry DMF and EtN*i*Pr_2 (13 µL, 3 equiv.), and the reaction mixture was sparged for 30 mins.

A separate vial was charged with the amine (1.6 equiv.) followed by 1 ml DMF and sparged with nitrogen for 30 mins.

Add the amine solution after sparge dropwise to the Ir-acid solution and stir for 16 h.

Following completion of the reaction, dilute the reaction mixture with EA (30 mL) and wash with $NaHCO_3$ (2x), water, 0.1 M HCl in brine (2x), and brine, before drying over sodium sulfate. The solution was concentrated under reduced pressure to give a yellow solid that can be purified by standard techniques.

Example Hyas/dione labeling procedure – Plate protocol

Labeling/lysis

To HeLa cells in 10 cm plates at 80% confluency in DMEM with **no** phenol red (Gibco) (4 mL) was added Ligand-PEG3-Ir (1-5 µM) (4 plates, **A**); Ligand-PEG3-Ir (1-5 µM) and ligand (10-50 µM) (4 plates, **B**); and DMSO. The plates were incubated at 37 °C for 3 hours and the media removed and replaced. Diazirine-PEG3-biotin was added (250 µM) and the plates incubated at 37 °C for an additional 20 minutes. The plates were subsequently irradiated (without the lid) in the bioreactor at 450 nM for 20 minutes. The media was removed, and the cells washed twice with cold DPBS (4 °C). The cells were resuspended in cold DPBS (4 °C), scraped, and transferred to a separate 15 mL falcon tube. The cells were pelleted (1000g for 5 minutes at 4 °C) and suspended in 1 mL of cold RIPA buffer containing PMSF (1mM) and cOmplete EDTA free protease inhibitor (Roche). The lysed cells were incubated on ice for 5–10 minutes and sonicated (35%, 5 x 5s with 30s rest). The lysate was then centrifuged at 15x1000g for 15 mins at 4 °C and the supernatant collected. The concentration of the cell lysate was measured by BCA assay (typically 1 to 1.5 mg/mL) and volumes adjusted accordingly to equal concentration. A sample was removed and stored at –20 °C for future analysis.

Streptavidin pull-down:

Magnetic Streptavidin beads (New England Biolabs S1420S) were removed (250 µL) and washed twice with RIPA (0.5 mL). The samples were incubated with the beads on a rotisserie at 4 °C overnight. The beads were pelleted on a magnetic rack and the supernatant removed. The beads were subsequently washed with 3 x 1% SDS in DPBS (0.5 mL), 3 x 1M NaCl in DPBS (0.5 mL), 3 x 10% EtOH in DPBS (0.5 mL). The samples were incubated with each wash for 5 minutes prior to pelleting. The beads were resuspended in RIPA buffer (300 µL) and transferred to a new 0.5 mL Lo-bind tube.

Following streptavidin enrichment, the samples can be either eluted for western blot analysis or processed for chemoproteomic analysis according to standard protocols.

Example Hyas labeling procedure – Tube protocol

Labeling/lysis

K562 cells in T75 flasks in IMDM were pelleted at 500g and resuspended in fresh IMDM and transferred to T25 flasks (1 per experimental replicate, approx. 20 M cells per flask) (4 mL). To these flasks was added Ligand-PEG3-Ir (1-5 µM) (**A**); Ligand-PEG3-Ir (1-5 µM) and ligand (10-50 µM) (**B**); and DMSO. The plates were incubated at 37 °C for 3 hours before the cells were pelleted and transferred to 1.5 mL Eppendorf tubes in 1 mL Diazirine-PEG3-biotin (250 µM) in HEPES buffered saline. The tubes were incubated at 37 °C for 20 minutes. The tubes were subsequently irradiated in the Photoreactor at 450 nM for 3 minutes. The cells were then pelleted at 500g, and the cells washed twice with cold DPBS (4 °C). The cells were then suspended in 1 mL of cold RIPA buffer containing PMSF (1mM) and cOmplete EDTA free protease inhibitor (Roche). The lysed cells were incubated on ice for 5–10 minutes and sonicated (35%, 5 x 5s with 30s rest). The lysate was then centrifuged at 15x1000g for 15 mins at 4 °C and the supernatant collected. The concentration of the cell lysate was measured by BCA assay (typically 1 to 1.5 mg/mL) and volumes adjusted accordingly to equal concentration. A sample was removed and stored at –20 °C for future analysis.

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