

b

React amino group of lysine in suc-KLLVY-AMC
with AMAS (NHS ester)

DMF with DIPEA

HPLC purify

Conjugate N-terminal cysteine of titin concatamers
with KLLVY-crosslinker (maleimide)

PBS pH 7.2

Remove excess peptide

Denature with GdnHCl and carboxymethylate internal
cysteines of titin domains with iodoacetic acid

Tris pH 8.9

Remove denaturant

Ubiquitinate *in vitro*

Ubc1, Uba1, Rsp5
pH 8.0

Monitor degradation by increase in fluorescence