HAT Assay

Stock solution

50 mM Tris, pH 8.0
10% glycerol

Washing solution

50 mM Tris, pH 8.0
10% glycerol
1 mM DTT
1 mM PMSF
1 mM Na-Butyrate

Assay buffer

Washing solution with 10 mM Na-Butyrate
for 92 µl:
85 µl washing solution
6 µl 0.5 M Na-Butyrate
1 µl 14C-Acetyl-CoA (50 mCi/mmol)

Protocol

- purify a HAT activity (IP, GST) according to standard procedures
- wash beads twice with washing buffer
- prepare assay buffer
- aspirate beads to a final volume of 20 µl
- add to every tube 10 µl of assay buffer and add additionally 5 - 20 µg of target proteins (eg. histones)
- incubate for 1 hour at 30 degrees in a waterbath, flick tubes every 5 minutes
- add 4 µl of 10X SDS-PAGE loading buffer
- cook, load onto 15% SDS-PAGE
- stain gel with Coomassie, destain
- incubate gel for 30 minutes in 1 M Na-Salicylate
- dry gel and expose for several days