IP/HAT assay

Lyse cells in 0.5% NP-40 lysis buffer

Preclear for 2 hours with protein A agarose

Incubate with antibody overnight at 4°C

Add protein A agarose and incubate for an additional 2 hours at 4°C

Wash immunoprecipitates 2 times with lysis buffer and 2 times with HAT assay buffer

Incubate beads in 30 µl HAT assay buffer, histones and [^{3}H]acetyl CoA at 30°C for **1 hr**

HAT assay buffer:

50 mM Tris-HCl (pH 8.0)
10 mM sodium butyrate
10% glycerol
1 mM DTT
1 mM PMSF

+ 25 µg crude histones
+ 50 nCi [^{3}H]acetyl-CoA

Spot 20 µl of reaction onto Whatman P-81 filter paper

Wash for 30 min with 0.2 M sodium carbonate buffer (pH 9.2) (Change buffer 2 times)

Dry filters and count in liquid scintillation counter