

# A rapid and sensitive assay for histone acetyl-transferase activity

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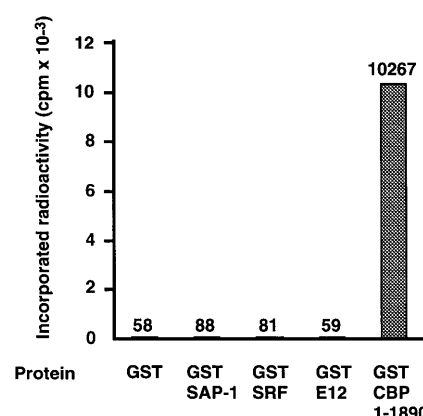
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## ABSTRACT

**Histone acetyl-transferases (HATs) seem to be key elements in the regulation of transcription. We have designed an enzymatic assay to quantify HAT enzymatic activity. In this assay, the substrate is a peptide corresponding to the 24 first amino acids of histone H4 which is coupled to biotin. After acetylation using [<sup>14</sup>C]acetyl-CoA, the peptide is purified on streptavidin beads and the associated radioactivity is measured. This assay is sensitive, rapid and convenient.**

Histone acetyl-transferases (HATs) and histone deacetylases appear to play a major role in the control of gene expression (1–3). In particular, some transcriptional co-repressors are associated with histone deacetylases (4–8), and several transcriptional co-activators are HATs (9–11). HAT activity can be detected *in vitro* by three different assays: an in-gel acetyl-transferase assay allows the rapid molecular characterisation of the enzymes (12); another assay, in which the histone substrate is acetylated with [<sup>14</sup>C]acetyl-CoA requires the resolution of the labelled product by SDS–PAGE, a procedure which is time consuming and poorly quantitative (10,11); finally, HAT activity can be quantified using a filter binding assay, in which the radioactivity transferred to histones is measured after adsorption onto a phosphocellulose filter (12,13). In this paper, we describe a rapid HAT assay which is quantitative, sensitive and shows a high signal-to-noise ratio.

A synthetic peptide corresponding to the first 24 amino acids of histone H4 (sequence SGRGKGGKGLGKGGAKRHRKVLRL) was coupled through a GSGS linker sequence to a biotin molecule. This peptide was used as a substrate in a HAT assay. Note that in order to broaden the range of detected enzymatic activities, a mixture of peptides corresponding to all four core histone tails could be used. After incubation in the presence of [<sup>14</sup>C]acetyl-CoA and a source of enzyme, the radiolabelled peptide was specifically retained on beads covered with streptavidin, which were subsequently counted in a liquid scintillation counter. A significant amount of radioactivity was incorporated into the peptide and retained on the beads when bacterially produced recombinant CBP (10,11) was used as a source of HAT enzyme (Fig. 1, GST–CBP, 1–1890). In contrast, very low counts were retained when similar amounts of the GST moiety of the



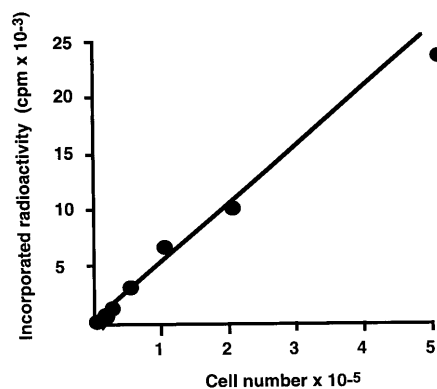
**Figure 1.** Specificity of the assay. cDNAs of interest were cloned into the relevant pGEX-2T vector (Pharmacia). Details of constructions are available upon request. Recombinant GST proteins were expressed and purified as reported by Kaelin *et al.* (14), with minor modifications. GST–CBP (5 pmol; 1–1890) recombinant protein or irrelevant proteins: GST, GST–SAP1, GST–SRF and GST–E12 were used in the HAT assay, and the incorporated radioactivity is shown. Numbers on top of the bars show the results in c.p.m.

GST–CBP fusion protein (GST) or other recombinant fusion proteins (GST–SAP1A, GST–SRF and GST–E12) were used, indicating that the retained radioactivity does not reflect a non-specific adsorption.

In order to assess the linearity of the response, samples containing cellular CBP immunoprecipitated from increasing numbers of cells were assayed. In the range tested, the dose response was linear (Fig. 2). Finally, we compared this assay with the other quantitative assay for HAT activity, the classical filter binding assay in which a mixture of histones were used (Table 1). Three distinct samples containing immunoprecipitated cellular CBP were compared in the two assays. For all samples, the signal-to-noise ratio was dramatically higher with the peptide–biotin conjugate than with the filter binding assay. Note that when the peptide was used as a substrate in filter binding assays instead of the histones, a good signal-to-noise ratio was also obtained, with only a 2-fold decrease in the sensitivity, as compared to the assay using the beads (data not shown). However, only the use of

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**Figure 2.** Linearity of the assay. CBP was immunoprecipitated from the indicated number of NIH3T3 cells, using standard procedures with the A-22 anti-CBP antibody (Santa Cruz): beads were washed twice with RIPA buffer [50 mM Tris pH 7.5, 150 mM NaCl, 1% NP-40, 0.5% sodium deoxycholate, 0.1% SDS, 1 mM EDTA and a cocktail of protease inhibitors (Boehringer)] and three times with HAT buffer (50 mM Tris pH 7.5, 1 mM EDTA and protease inhibitors). Immunoprecipitates were used in a HAT assay: the H4 biotinylated peptide was purchased from Chiron (synthesis protocol available upon request); the peptide (30  $\mu$ M final) was mixed with samples containing the enzymes immobilised on beads in 30  $\mu$ l of HAT buffer supplemented with 100 nCi of [<sup>14</sup>C]acetyl-CoA (63 mCi/mmol, ICN) and incubated at 30°C for 45 min. After centrifugation, supernatants were incubated in HAT buffer (500  $\mu$ l) with 10  $\mu$ l of pre-washed streptavidin-agarose beads (Sigma) for 20 min at 4°C on a rotating wheel, beads were washed twice with RIPA buffer, mixed with 2 ml of scintillation liquid (Hionic, Packard) and counted using a  $\beta$  counter (LKB). The filter binding assay was performed as described in (10).

the biotin-streptavidin system allows the direct measurement of HAT activity from whole cell lysates, results of which would be difficult to interpret using the filter binding assay. Furthermore, the assay using the streptavidin beads is more rapid and convenient.

In conclusion, this assay is quantitative and sensitive. In addition, the same principle could also be applied to measure histone deacetylase activities.

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**Table 1.** Comparison between two quantitative HAT assays

Experiment	Sample	Biotinylated peptide assay (c.p.m) <sup>a</sup>		Filter binding assay (c.p.m) <sup>a</sup>	
1	Irrelevant <sup>b</sup>	273		876	
	Sample <sup>c</sup>	17 250	(63) <sup>d</sup>	1 631	(1.9)
2	Irrelevant <sup>e</sup>	507		3 467	
	Sample 1	57 653	(113.7)	11 500	(3.3)
	Sample 2	64 022	(126.3)	13 074	(3.7)
	Sample 3	74 910	(147.7)	14 465	(4.2)

The same immunoprecipitated samples were tested using the biotinylated peptide or the filter binding assay, using a mixture of histones.

- <sup>a</sup>Incorporated radioactivity.
- <sup>b</sup>Irrelevant antibody (anti-HA epitope).
- <sup>c</sup>Anti-CBP antibodies.
- <sup>d</sup>Signal-to-noise ratio between brackets.
- <sup>e</sup>Irrelevant antibody (anti-myogenin).

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