Human AID recombinant protein

Catalog #	Lot No	Product Name	Size	
GU/1/2014	2/2014/09/16	Human AID recombinant protein	0.5 U/µl (20 µl)	
GU/2/2014	2B/2014/09/16	Reaction Buffer 10×	1ml	

SOURCE:

Human activation-induced cytidine deaminase (hAID) fully active recombinant fusion protein is partially purified from *E. coli* containing a recombinant plasmid harboring the human AID gene.

UNIT DEFINITION:

One Unit (1 U) is defined as the amount of the enzyme required to deaminate 15 fmole of an oligonucleotide containing a single dC residue in 1h at 37°C. Enzymatic efficiency of hAID depends on the oligonucleotide structure at 37°C and accessibility of dC residue for the enzyme.

SUBSTRATE SPECIFICITY:

hAID enzyme deaminates deoxycytidine to deoxyuridine in a single-stranded DNA (ssDNA), preferable within WRCY hot spot motif (W = A/T; R = A/G; Y = C/T). The optimal temperature for the enzyme is 37°C. The enzyme lacks AP lyase or endonuclease activity.

ASSAY CONDITIONS & ANALYSIS:

Deoxycytidine deamination reaction

40 fmol (20 000 cpm) of 5'-radiolabeled 80-nucleotide single-stranded oligonucleotide (with a single deoxycytidine residue at position 40) is incubated for 30 min at 37°C with 1U or 2U of human AID recombinant protein in the Reaction Buffer 1X in a final volume of 10 µl. In a negative control (C-), the 80-nucleotide ssDNA with a single dC residue in the central position is incubated with the Reaction Buffer 1× only. In a positive control (C+), an 80-nucleotide ssDNA with a single dU residue located in the central position and without any dC residue is analogously used.

Deoxyuridine detection assay

Two units of *E. coli* uracil-DNA glycosylase (UDG, Thermo Scientific) are added directly to the reaction mixture and incubation is continued for 30 min at 37°C. UDG catalyzes the hydrolysis of the N-glycosylic bond between uracil and sugar, leaving an abasic site. To cleave the abasic site, fresh 2M NaOH is added to a final concentration of 200 mM and the reaction mixture is heated for 15 minutes

at 80°C. Products of the reaction are analyzed by electrophoresis in 15% denaturing polyacrylamide gel and visualized using a PhosphorImager. In the presence of deaminase activity, it is expected to observe a 40-nucleotide-long product.

The UDG-coupled deamination assay for measuring AID activity on dC has been described previously (Sohail et al, 2003).

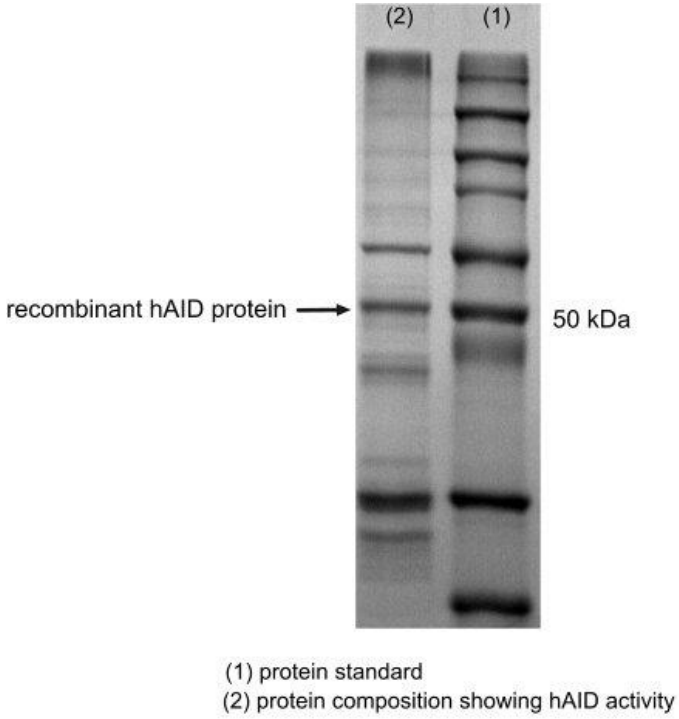

Figure 1. SDS-PAGE analysis of partially purified human AID recombinant protein (12% Tris-Glycine gel).

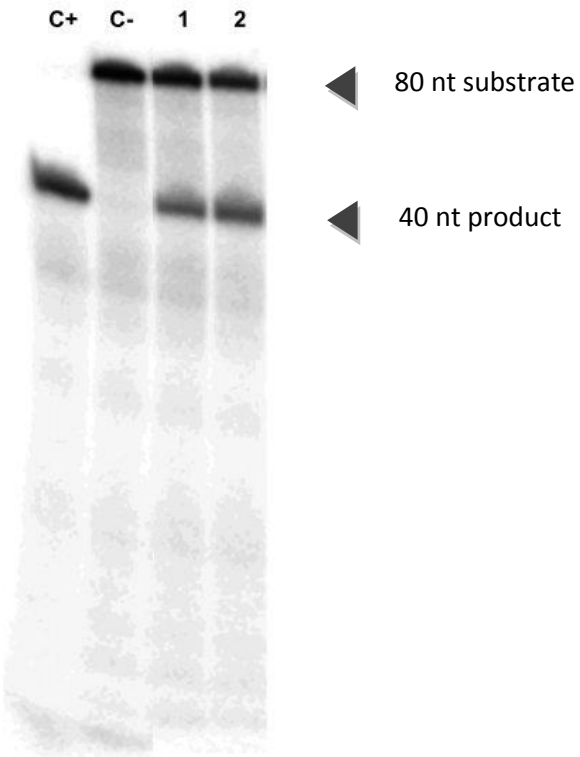

Figure 2. Electrophoretic analysis of the products of the UDG-coupled deamination assay; activity test of hAID recombinant protein. (1) 1U human AID recombinant protein. (2) 2U human AID recombinant protein. C+/C- positive/negative control (description in the text).

REACTION BUFFER 10×:

500 mM Tris-HCl, 10 mM DTT, pH 8.0

STORAGE BUFFER:

50 mM Tris-HCl, 1 mM DTT, 50% glycerol (w/v), pH 8.0

STORAGE

Store at -20°C in a manual defrost freezer. Avoid repeated freeze-thawing. Enzyme may be diluted in 1× Reaction Buffer for immediate use. Human AID recombinant protein in storage buffer can survive for at least 3 months with less than 10% loss in activity.

REFERENCES:

1. Sohail A, Klapacz J, Samaranayake M, Ullah A, Bhagwat AS; Human activation-induced cytidine deaminase causes transcription-dependent, strand-biased C to U deaminations; Nucleic Acids Res. 2003 Jun 15;31(12):2990-4.