

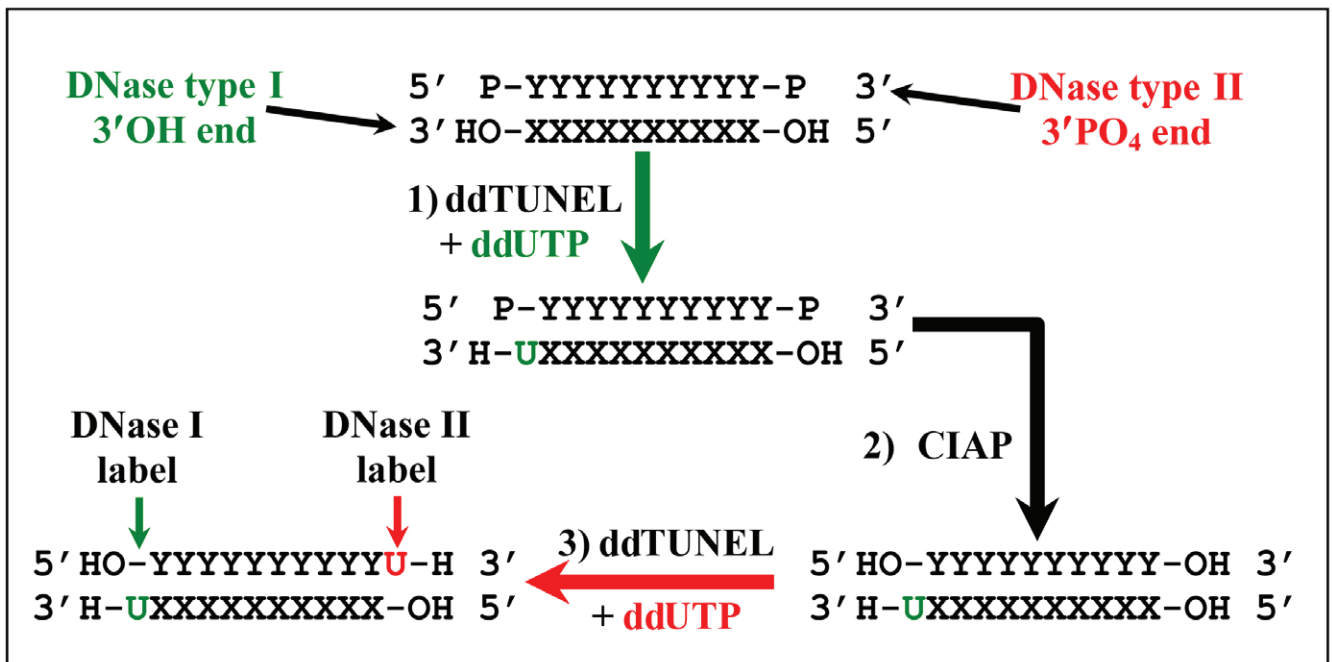
Supplementary Material For:

Quantification of DNase type I ends, DNase type II ends, and modified bases using fluorescently labeled ddUTP, terminal deoxynucleotidyl transferase, and formamidopyrimidine-DNA glycosylase

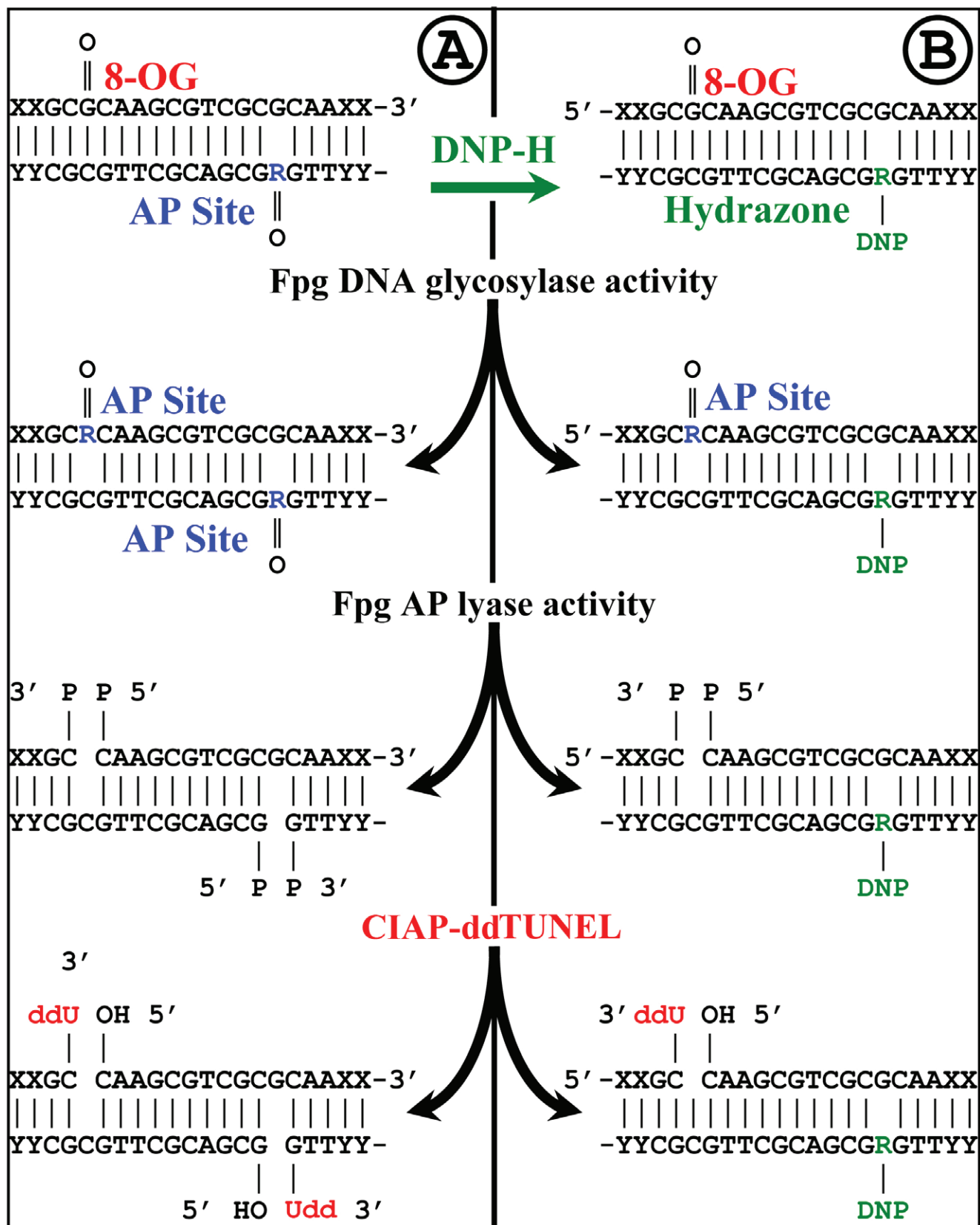
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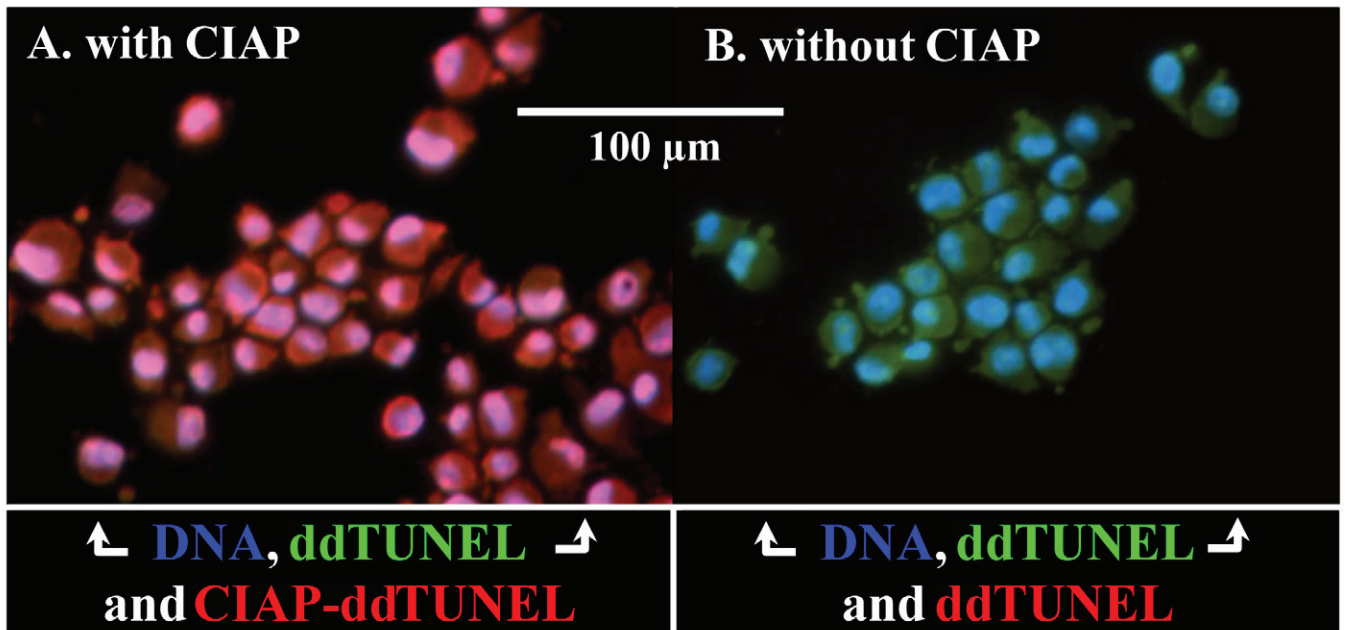
Keywords: TUNEL; ddUTP; formamidopyrimidine-DNA glycosylase; DNase; apoptosis; mammary gland; U87; chemotherapy



Supplementary Figure S1. Use of the ddTUNEL and CIAP-ddTUNEL to measure 3'OH and 3'PO₄ DNA ends. A representative damaged section of double-stranded DNA—with both DNase type I and type II ends—is shown. After a round of ddTUNEL, using a green-labeled ddUTP, all 3'OH ends are labeled (1). All 3'PO₄ DNase type II ends are converted into ddTUNEL-positive 3'OH ends using CIAP (2), and these are labeled with red-labeled ddUTP (3) in a second round of ddTUNEL.



Supplementary Figure S2. Use of Fpg-ddTUNEL and DNP-H to discriminate between modified bases and AP sites. (A) A representative damaged section of double-stranded DNA with an oxidized guanine (G = O) and an AP site (R = O). The sample can be incubated with DNP-H (B), to convert the AP site into a hydrozone, which is not a substrate for Fpg. The samples are treated with Fpg, followed by CIAP, to generate 3'OH ends and then with ddTUNEL. In samples not treated with DNP-H (A), both the 8-OG and AP sites are labeled with ddUTP. In the derivatized sample (B), only 8-OG is labeled by ddTUNEL; however, the presence of the ribose-hydrazone can be independently labeled using an anti-DNP antibody.



Supplementary Figure S3. Labeling DNase type II–treated U87 cells using ddTUNEL and CIAP-ddTUNEL to measure 3'OH and 3'PO₄. U87 cells were grown on slides, fixed, permeabilized, washed, and then treated with DNase type II for 2 h. The 3'OH ends were labeled green using ddTUNEL (biotin-ddUTP/FITC-avidin). The levels of 3'OH ends in DNase type II–treated cells were identical to those of control cells incubated with the enzyme omitted from the DNase type II buffer (results not shown). After ddTUNEL, half of the samples were incubated with CIAP and the other half with only CIAP buffer. The CIAP-positive and -negative samples were then incubated with a second ddTUNEL assay mixture (biotin-ddUTP/Texas Red–avidin). Only cells that were incubated with CIAP (A) were labeled in the second round of ddTUNEL. Cells in which 3'PO₄ ends were not converted in vitro into 3'OH ends (B) were only stained in the initial ddTUNEL round.