

PROTEOMICS

Supporting Information for Proteomics

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Tyrosine 656 in topoisomerase II β is important for the catalytic activity of the enzyme: Identification based on artifactual +80-Da modification at this site

Supplemental Method

Metabolic labeling with [³²P]orthophosphoric acid and phosphopeptide mapping

Log phase cultures of BJ201 yeast cells expressing WT or mutant topo II β were first incubated in medium lacking phosphate and then incubated with carrier free [³²P]orthophosphoric acid (250 μ Ci/ml) as described previously [29]. WT or mutant topo II β purified by immunoprecipitation or Ni-NTA agarose chromatography and SDS-PAGE was transferred to nitrocellulose membrane and digested with trypsin as described earlier. The tryptic digests were subjected to 2D-phosphopeptide mapping involving electrophoresis with pH 1.9 buffer (88% formic acid/glacial acetic acid/deionized water – 1/3.1/36, v/v) in the horizontal dimension and chromatography in the vertical dimension using phosphochromatography buffer, (*n*-butyl alcohol/pyridine/acetic acid/deionized water – 5/3.3/1/4, v/v) or isobutyric acid buffer: isobutyric acid/*n*-butanol/pyridine/acetic acid/deionized water - 32.9/1/2.5/1.5/14.7, v/v)