Star Activity

Under non-standard reaction conditions, some restriction enzymes are capable of cleaving sequences which are similar, but not identical, to their defined recognition sequence. This altered specificity has been termed “star activity". It has been suggested that star activity is a general property of restriction endonucleases (1) and that any restriction endonuclease will cleave noncanonical sites under certain extreme conditions, some of which are listed below. Although the propensity for star activity varies, the vast majority of enzymes from New England Biolabs will not exhibit star activity when used under recommended conditions in their supplied NEBuffers. If an enzyme has been reported to exhibit star activity, it will be indicated in the product entry found in the catalog, on the datacard or on our web site.

The manner in which an enzyme's specificity is altered depends on the enzyme and the reaction conditions which induce star activity. The most common types of altered activity are single base substitutions, truncation of the outer bases in the recognition sequence, and single-strand nicking (2). Some enzymes exhibit relaxation of sequence specificity under standard conditions and in the presence of the cognate site are capable of cleaving non-cognate (secondary) sites (3).

What is Restriction Enzyme Star Activity?

Learn what Star Activity is, why it is detrimental to accurate restriction enzyme digestion, and how NEB's HF enzymes are engineered to avoid it.

Conditions that Contribute to Star Activity	Steps that can be Taken to Inhibit Star Activity
High glycerol concentration (> 5% v/v)	Restriction enzymes are stored in 50% glycerol, therefore the amount of enzyme added should not exceed 10% of the total reaction volume. Use the standard 50 µl reaction volume to reduce evaporation during incubation.
High concentration of enzyme/µg of DNA ratio (varies with each enzyme, usually 100 units/µg)	Use the fewest units possible to achieve digestion. This avoids overdigestion and reduces the final glycerol concentration in the reaction.
Non-optimal buffer	Whenever possible, set up reactions in the recommended buffer. Buffers with differing ionic strength and pH may contribute to star activity.
Prolonged reaction time	Use the minimum reaction time required for complete digestion. Prolonged incubation may result in increased star activity, as well as evaporation.
Presence of organic solvents [DMSO, ethanol (4), ethylene glycol, dimethylacetamide, dimethylformamide, sulphalane (5)]	Make sure the reaction is free of any organic solvents, such as alcohols, which might be present in the DNA preparation.
Substitution of Mg^{2+} with other divalent cations (Mn^{2+}, Cu^{2+}, Co^{2+}, Zn^{2+})	Use Mg^{2+} as the divalent cation. Other divalent cations may not fit correctly into the active site of the restriction enzyme, possibly interfering with proper recognition.

Note: The relative significance of each of these altered conditions will vary from enzyme to enzyme.

New England Biolabs recommends setting up restriction enzyme digests in a 50 µl reaction volume. However, different methods may require smaller reaction volumes. When performing restriction enzyme digests in smaller reaction volumes, extra care must be taken to follow the steps listed above to avoid star activity. Alternatively, using our new line of High-Fidelity (HF™) Restriction Enzymes will allow some flexibility in reaction setup.

References:

1. Nasri, M. and Thomas, D. (1986) *Nucleic Acids Res.* 14, 811. PMID: 3003698
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3. Bitinaite, J. and Schildkraut, I. (2002) *Proc. Natl. Acad. Sci USA* 99, 1164-1169. PMID: 11818524
4. Nasri, M. and Thomas, D. (1987) *Nucleic Acids Res.* 15, 7677. PMID: 2823216
5. Tikchinenko, T. I. et al. (1978) *Gene.* 4, 195-212. PMID: 33871