Resolve-It™ Kit

Sequence-Specific DNA Ligands
Cat. No. MB-1401

Sequence-specific DNA ligands bind to DNA in a sequence-dependent manner and retard the electrophoretic migration of the DNA. This ability allows for the separation of DNAs of similar size but different sequence in an agarose gel. Applications such as differential display frequently generate multiple products with similar electrophoretic mobility. The band of interest needs to be isolated from the others before subsequent sequencing and analysis can occur. After excision of the band of interest from a standard gel, DNA(s) from this band are electrophoresed and separated in a gel containing one of the DNA ligands. Finally, bands may be excised and applied to a gel containing the other DNA ligand for confirmation of homogeneity before cloning or sequencing.

Since it is possible for DNAs of different sequence to contain a similar number of ligand binding sites, two ligands, each with a different mechanism of binding, are provided to increase the likelihood of obtaining separation. The Resolve-It™ Kit contains two ligands. AT-Yellow™, a bisbenzimide-PEG conjugate, binds to the DNA minor groove at regions of four consecutive A•T base pairs. GC-Red™, a 10-phenyl neutral red-PEG conjugate, intercalates within G•C rich regions. The PEG conjugated to each ligand increases the coefficient of friction during electrophoresis, causing the differential migration of DNAs depending on the amount of ligand bound.

Caution: The toxicity of the ligands in the Resolve-It™ Kit is unknown. However, since these products bind DNA, use gloves and other appropriate safety measures.

Instructions for use:

Reconstitution: Add 1.4 ml of H_20 to the AT-Yellow™ and mix. Add 1.4 ml of H_20 to the GC-Red™ and mix. The reconstituted ligands contain 0.08% sodium azide as a preservative.

Storage: Store solid and reconstituted ligands at 4 °C in the dark. Do not freeze.

Gel preparation: Prepare an agarose gel in 0.5x TBE or 1x TAE*, pH 7.5. TBE and TAE made using most common recipes are at a higher pH and must be adjusted to pH 7.5. After heating, cool the gel to about 65 °C, then add either 7 µl of AT-Yellow™/ml of agarose or 7 µl of GC-Red™/ml of agarose. Mix and pour into the gel-casting tray. Note: DNA-containing gel slices excised from a previous, standard gel can be placed in the gel tray and surrounded by the molten, DNA ligand-containing agarose.

* 0.5x TBE is 44.5 mM Tris, 44.5 mM boric acid, 1 mM EDTA, pH 7.5; 1x TAE is 40 mM Tris, 20 mM acetic acid, 1 mM EDTA, pH 7.5.

Electrophoresis: Immediately before loading, submerge the gel in the buffer used in gel preparation. Avoid soaking the gel for an extended time before loading samples because the DNA ligands will slowly diffuse from the gel. Load samples. Apply ≤ 3.5 V per centimeter between electrodes. Higher voltages may overheat the gel and reduce the efficiency of DNA ligand binding.

Visualization: After electrophoresis, it may be possible to view DNA bands in gels containing AT-Yellow™ with UV excitation due to the fluorescence of the AT-Yellow™ bound to the DNA(s). For greater sensitivity, soak the gel in a solution of 0.5 µg/ml ethidium bromide in distilled water for 30 minutes. De-stain for several minutes before visualizing with UV light. Gels containing GC-Red™ may require a longer exposure to ethidium bromide because the GC-Red™ may occupy ethidium bromide binding sites and needs to be displaced. GC-Red™ is not fluorescent.

Reference: Muller W. et al. 1981. Polyethylene glycol derivatives of base and sequence specific DNA ligands: DNA interaction and application for base specific separation of DNA fragments by gel electrophoresis. Nucleic Acids Res. 9(1):95-119.

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Vector Laboratories, Inc., 30 Ingold Road, Burlingame, CA 94010 U.S.A.,
Tel: (650) 697-3600 • Fax: (650) 697-0339 • Email: vector@vectorlabs.com • Web: www.vectorlabs.com