Kinetoplast DNA (kDNA) and markers

(isolated from *Crithidia fasciculata*)

Product Description (Product numbers K2001, K1002, K2003, K4004, KL100, KD100)

kDNA is purified from *Crithidia fasciculata* using the method described in Shapiro *et al* (1999).
Decatenated kDNA markers are prepared by treating kDNA with *E.coli* topo IV and then mixing with loading buffer.
Linear mini-circle markers are prepared by treating kDNA with the restriction enzyme NdeI to linearise the mini-circles and then mixing with loading buffer.
Store kDNA at 4 °C. Stable for 12 months. Markers should be stored at -20 °C.
For *in vitro* laboratory research use only.

kDNA

Supplied at 100 ng/µl in 10 mM Tris-HCl (pH 8.0), 1 mM EDTA.

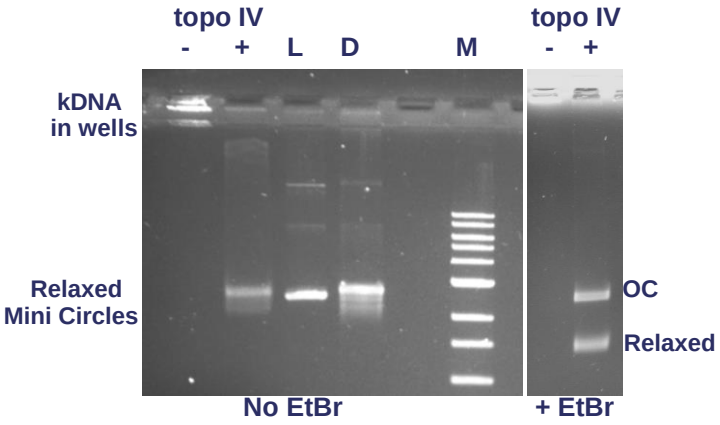
The kDNA should be stored at 4 °C.

Markers

1. Linearised kDNA (mini-circles).
2. Decatenated kDNA (mini-circles).
Both supplied at 50ng/ul in 50 mM Tris-HCl (pH 8.0), 5 mM EDTA, 20 % (w/v) sucrose, 0.25mg/ml bromophenol blue.

Decatenation Assay

1 U of topoisomerase IV will decatenate 200 ng of kDNA when incubated in 1X Assay Buffer in a total reaction volume of 30 µl at 37 °C for 30 minutes.
Gels can be run in the presence or absence of intercalators (eg ethidium bromide (EtBr) or chloroquine which will resolve nicked OC DNA from relaxed topoisomers).
Markers are 2µl of mini-circles linearised from kDNA (L) and 2µL of decatenated mini-circles (D). M= Kb ladder. The linearised mini-circles are ~2.3 Kb in size.



Quality Control

kDNA is purified to give a OD_{260} to OD_{280} ratio of 1.8-1.9 with no detectable free mini-circles when catenated kDNA is run on a 1 % (w/v) agarose gel. Each batch is tested for decatenation with purified *E.coli* topo IV.

Reference

Shapiro, T.A., Klein, V.A. and Englund, P.T. (1999). Isolation of kinetoplast DNA, in *DNA Topoisomerase Protocols* Vol. I (Bjornsti, M-A and Osheroff, N. 1999). Humana Press, Totowa, N.Jersey pp. 61-68