

**ISOLATION OF CELL NUCLEI FROM ANIMAL TISSUES BY THE CITRATE METHOD**

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The citrate method is a reliable and short procedure that provides cell nuclei with intact genomic DNA. The method is especially useful for the isolation of high molecular weight DNA using DNAzol® (Cat. No. DN 127). Tissues or cells are homogenized or lysed in a solution containing sodium citrate and Triton X-100. Triton X-100 dissolves cytoplasmic membranes and citrate ions chelate magnesium and other divalent ions, thus inhibiting DNase activity. Following homogenization, nuclei are separated from the homogenate by a low speed centrifugation. The citrate method removes soluble proteins from the cell nuclei and should not be used in conjunction with functional assays such as nuclear runoff transcription.

**REAGENTS**

Sodium citrate, Triton X-100. Both reagents should be ACS grade or molecular biology grade.

Lysis solution: 40 mM sodium citrate and 1% Triton X-100.

**PROTOCOL**

1. Homogenize tissue in a glass-Teflon homogenizer using 1.0 ml of lysing solution per 100 mg of tissue. For tissues containing a high level of fat use 2 - 3 ml of lysing solution per 100 mg of tissue. Following homogenization, store the homogenization tube vertically for 30 sec to allow large clumps and tissue fragments to settle at the bottom of the homogenate. Transfer the homogeneous nuclear suspension by pipeting it into a clean tube.
2. Centrifuge the homogenate at 500 g for 5 minutes. Remove the supernatant and suspend the nuclear pellet by gentle vortexing or pipetting in a small amount of water. The water volume should not exceed 5% of the volume of DNAzol used for lysis of the nuclei (next step).
3. Lyse the nuclei by adding 1.0 ml of DNAzol per a maximum of  $3 \times 10^7$  nuclei. Mix DNAzol with the nuclei by inverting the tube. If necessary, dissolve the nuclear suspension by gentle pipetting.

**NOTES**

1. Addition of DNAzol to the nuclear pellet before suspending the pellet in water results in the formation of a clump that is difficult to dissolve.
2. This procedure can be used for isolation of nuclei from fresh or frozen tissues. Hard to homogenize tissues, such as muscles, placenta and skin should be powdered in liquid nitrogen before homogenization. Alternatively, these tissues can be minced into very small fragments using scissors. Mincing of tissues results in a lower yield of nuclei than tissues pulverized in liquid nitrogen.
3. The citrate method also can be used for the isolation of nuclei from cultured cells. However, the isolation of nuclei is not necessary for the isolation of high quality genomic DNA using DNAzol.
4. The quality of nuclei can be evaluated microscopically following methylene blue staining of the nuclei. Place a drop of the nuclear suspension on a glass slide and mix with a small amount of Methylene Blue Stain (Cat. No. MB 119).

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