Benchmarks


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Heriberto Correia, José Rivero and Flor Herrera Universidad de Carabobo Maracay, Venezuela

Isolation of Nuclear Proteins from Human Brains

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DNA-protein interaction is an important initial step of transcription. Many methods, including mobility shift and DNA footprinting, have been developed to explore the interaction of the DNA-protein complex in the initiation of transcription (1). The first key step in these methods is to obtain an adequate number of nuclear proteins that include transcription factors. During the preparation of nuclear proteins, caution should be taken to prevent their degradation by proteases, and other appropriate environmental conditions are necessary to maintain the proteins’ stability (2). Several procedures have been developed successfully in isolating nuclear proteins from various cell lines and tissues for the study of protein-DNA interaction (3–5). However, some of these procedures are time-consuming, requiring ultracentrifugation, sucrose gradient enrichment of nuclei or dialysis. Such prolonged operations may affect the quality of nuclear proteins, especially in brain tissue that contains a high number of lipids interspersed with relatively few neural cells. We describe a simple, rapid and reproducible method for isolating nuclear proteins from human brains that yields several common transcription factors.

Approximately 0.5 g of brain (cerebral) tissue (frozen in liquid nitrogen for up to 15 years) is rapidly triturated with a pestle and a mortar. One milliliter of a buffer, containing 10 mM HEPES (pH 7.9), 10 mM KCl, 0.1 mM EDTA, 0.1 mM EGTA, 1 mM dithiothreitol (DTT), 0.5 mM phenylmethylsulfonyl fluoride (PMSF) and 0.1% (vol/vol) leupeptin, is added to the powdered tissue. The mixture is transferred to a Dounce manual-type tissue grinder and homogenized with ten strokes. Twenty microliters of 10% Nonidet® P-40 (NP40) are added to the mixture (final concentration 0.5%) and chilled on ice for 15 min. The mixture is then homogenized with an additional five strokes and microcentrifuged at 1000× g for 5 min. Three layers of the
**Figure 1.** Mobility shift assay of human brain nuclear protein binding to oligonucleotides. Brain nuclear proteins or HeLa cell nuclear proteins (Promega, Madison, WI, USA) are added to 1 µg of poly(dI:dC) (Pharmacia Biotech, Piscataway, NJ, USA), and this mixture is added to each 32P-labeled (250,000 cpm) oligonucleotide of the following consensus sequences: AP1: 5′-CAGGATGTTCTAGCTACT-3′; CREB: 5′-AGAGATTGCCTGACGTCAGAGAGCTAG-3′; GRE: 5′-TCGACTGAACAGAAGAAGACT-3′; NF-κB: 5′-AGTTGAGGGGACTTTCCCAGGC-3′; STAT-3: 5′-TTCTGGGCCTTCTGGGGCGGGGCGAGC-3′ (Promega) and STAT-3: 5′-TTCTGGGAGATTTCTGGGCCGT-3′ (Santa Cruz Biotechnology, Santa Cruz, CA, USA). The mixtures are incubated for 20 min at room temperature in a binding buffer [20 mM Tris-HCl (pH 7.9), 2 mM MgCl2, 50 mM KCl, 1 mM EDTA, 2 mM DTT, 10% glycerol, 0.1 ng poly(dI:dC)] in a total volume of 20 µL. The resultant oligonucleotide-protein complex is resolved by electrophoresis (180 V for 3–4 h) on a 6% low cross-linked, non-denatured polyacrylamide gel with 0.5x TBE buffer (0.0045 M Tris-borate, pH 8.0, 1 mM EDTA). (A) Lanes 1–5 contain 12 µg brain nuclear proteins and the various labeled oligonucleotide probes; lanes 6–10 contain only the various labeled oligonucleotide probes; lanes 6–10 contain only the various labeled oligonucleotide probes; lanes 6–10 contain only the various labeled oligonucleotide probes; lanes 6–10 contain only the various labeled oligonucleotide probes; lanes 6–10 contain only the various labeled oligonucleotide probes. (B) Lanes 1 and 7 contain 2 µg HeLa cell nuclear proteins; lanes 2 and 8–10 contain 2 µg brain nuclear proteins; lanes 5 and 11 contain 2 µg bovine serum albumin (BSA); and lanes 6 and 12 contain no added protein. Lanes 1–6 contain labeled NF-κB probe with lanes 3 and 4 containing 10-fold excess of each unlabeled NF-κB probe and unlabeled STAT-3 probe, respectively. Lanes 7–12 contain labeled STAT-3 probe with lanes 9 and 10 containing 10-fold excess of each unlabeled STAT-3 probe and unlabeled STAT-3 (mutant) (5′-TTCTGCGCCGT-3′) probe, respectively (Santa Cruz Biotechnology).