

Online Resource 1

Detection of stress-inducible Hsp70 (HspA1) by dot blot immunoassay. Culture medium was collected from control cultures (incubation at 37°C) and at 24, 48 or 72 hr following 30 min heat shock at 43°C. Concentration of Hsp70 was determined by immunoassay against a standard curve of recombinant human Hsp70 (Assay Design) using a Bio-dot microfiltration system (Bio Rad) according to instructions. Briefly, 1.5 mL of medium was collected from each culture (18 mm coverslip, maintained in 12-well culture dishes) and applied directly to pre-wetted 0.2 µm nitrocellulose membranes by vacuum filtration. Membranes were blocked with 5% milk in Tris buffer (pH 7.4) for 1 hr at R.T. and incubated with primary antibody against Hsp70 (Stressgen; SPA 810; 1:1000) overnight at 4°C and HRP-conjugated secondary antibody for 1 hr at R.T. Blots were developed as described in Materials and methods.

Expression of the protein chaperone, clusterin, in spinal cord cells constitutively and following cellular stress, and upregulation by treatment with Hsp90 inhibitor. Cell Stress and Chaperones.

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