

MONOCLONAL ANTIBODY

# Anti-DJ-1

| Code No. | Clone | Subclass   | Quantity | Concentration |
|----------|-------|------------|----------|---------------|
| M043-3   | 3E8   | Mouse IgG1 | 100 µg   | 1 mg/mL       |

**BACKGROUND:** DJ-1 (PARK7/CAP1/RS) was originally cloned as a putative oncogene capable of transforming NIH/3T3 cells in cooperation with H-ras, a protein expressed in sperm, and a regulator of RNA-protein interactions. DJ-1 has also been isolated as a gene associated with autosomal early-onset Parkinson's disease (PD). Taken together, DJ-1 appears to be involved in diverse biological processes. First, several lines of evidence suggest that DJ-1 plays a role in the oxidative stress response. In cultured mammalian cells, DJ-1 quenches reactive oxygen species and is converted into a variant with a more acidic isoelectric point. Therefore, DJ-1 protects against reactive oxygen species-induced cell death, and its suppression with small interfering RNA (siRNA) sensitizes cells to such insults. Second, DJ-1 modulates transcription through interaction with DJ-1-binding protein as well as with protein inhibitor of activated STAT (PIAS). The latter modulates the activity of various transcription factors. Third, DJ-1 has been recognized as a regulatory subunit of an RNA-binding protein. Fourth, DJ-1, which is structurally related to the molecular chaperone Hsp31, may have chaperone activity itself, preventing heat-induced aggregation of substrate proteins, including  $\alpha$ -synuclein. In addition, several lines of evidence suggest that DJ-1 plays a role in human tumorigenesis. First, breast cancer patients have elevated levels of circulating DJ-1 and anti-DJ-1 autoantibodies compared to healthy and non-breast cancer patients. Secondly, DJ-1 protein is increased in primary non-small cell lung carcinoma samples. Thirdly, treatment of cells from the human lung cancer cell line NCI-H157 with paclitaxel and MEK inhibitor U0126 leads to a decrease in DJ-1 protein expression.

**SOURCE:** This antibody was purified from mouse ascites fluid using protein A agarose. This hybridoma was established by fusion of mouse myeloma cell P3U1 with Balb/c mouse splenocyte immunized with the recombinant full-length human DJ-1 (1-187 aa).

**FORMULATION:** 100 µg IgG in 100 µL volume of PBS containing 50% glycerol, pH 7.2. No preservative is contained.

**STORAGE:** This antibody solution is stable for one year from the date of purchase when stored at -20°C.

**REACTIVITY:** This antibody reacts with human DJ-1 (28 kDa) on Western blotting.

**APPLICATION:**

Western blotting: 1-10 µg/mL for chemiluminescence detection system

Immunohistochemistry: Not tested

Immunocytochemistry: Not tested

Immunoprecipitation: Not tested

Flow cytometry: Not tested

Detailed procedure is provided in the following **PROTOCOL.**

**SPECIES CROSS REACTIVITY:**

| Species          | Human              | Mouse   | Rat  |
|------------------|--------------------|---------|------|
| Cells            | Jurkat, Raji, HeLa | NIH/3T3 | PC12 |
| Reactivity on WB | +                  | -       | -    |

**INTENDED USE:**

For Research Use Only. Not for use in diagnostic procedures.

**REFERENCES:**

- 1) Brandopadhyay, R., *et al.*, *Brain* **127**, 420-430 (2004)
- 2) Nagakubo D., *et al.*, *Biochem. Biophys. Res. Comm.* **231**, 509-513 (1997)

This clone 3E8 is used as a de facto standard antibody for every researcher in the world. This hybridoma 3E8 was originally established by the collaboration with Prof. Hiroyoshi Ariga (Hokkaido University) and MBL.

**PROTOCOL:**

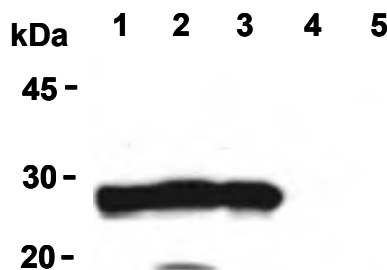
**SDS-PAGE & Western Blotting**

- 1) Wash the cells 3 times with PBS and suspend with 10 volume of cold Lysis buffer (50 mM Tris-HCl, pH 7.2, 250 mM NaCl, 0.1% NP-40, 2 mM EDTA, 10% glycerol) containing appropriate protease inhibitors. Incubate it at 4°C with rotating for 30 minutes, then sonicate briefly (up to 10 seconds).
- 2) Centrifuge the tube at 12,000 x g for 10 minutes at 4°C and transfer the supernatant to another tube. Measure the protein concentration of the supernatant and add the Lysis buffer to make 8 mg/mL solution.
- 3) Mix the sample with equal volume of Laemmli's sample buffer.
- 4) Boil the samples for 2 minutes and centrifuge. Load 10 µL

of the sample per lane in a 1 mm thick SDS-polyacrylamide gel for electrophoresis.

- 5) Blot the protein to a polyvinylidene difluoride (PVDF) membrane at 1 mA/cm<sup>2</sup> for 1 hour in a semi-dry transfer system. (Transfer Buffer: 25 mM Tris, 190 mM glycine, 20% MeOH). See the manufacture's manual for precise transfer procedure.
- 6) To reduce nonspecific binding, soak the membrane in 10% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature, or overnight at 4°C.
- 7) Incubate the membrane with primary antibody diluted with PBS, pH 7.2 containing 1% skimmed milk as suggest in the **APPLICATIONS** for 1 hour at room temperature. (The concentration of antibody will depend on condition.)
- 8) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 minutes x 6 times).
- 9) Incubate the membrane with the 1:10,000 HRP-conjugated anti-mouse IgG (MBL; code no. 330) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature.
- 10) Wash the membrane with PBS-T (5 minutes x 6 times).
- 11) Wipe excess buffer on the membrane, then incubate it with appropriate chemiluminescence reagent for 1 minute. Remove extra reagent from the membrane by dabbing with paper towel, and seal it in plastic wrap.
- 12) Expose to an X-ray film in a dark room for 5 minutes. Develop the film as usual. The condition for exposure and development may vary.

(Positive controls for Western blotting; Jurkat, Raji, HeLa)



**Western blot analysis of DJ-1 expression in Jurkat (1), Raji (2), HeLa (3), NIH/3T3 (4) and PC12 (5) using M043-3.**

**RELATED PRODUCTS:**

- CY-9050 Human DJ-1 ELISA Kit \*
- K0162-3 Anti-Cyclin A (E23.1)
- K0163-3 Anti-Cyclin A (E67.1)
- K0163-6 Biotin labeled Anti-Cyclin A (E67.1)
- K0128-3 Anti-Cyclin B1 (V152)
- K0164-3 Anti-Cyclin B1 (V92.1)
- K0189-3 Anti-Cyclin B2 (X121.10)
- 553 Anti-Cyclin D1 (polyclonal)
- MD-17-3 Anti-Cyclin D1 (5D4)
- MD-17-3H Anti-Cyclin D1 (5D4)
- K0062-3 Anti-Cyclin D1 (DCS-6)

- K0063-3 Anti-Cyclin D2 (DCS-3)
- K0064-3 Anti-Cyclin D2 (DCS-5)
- K0013-3 Anti-Cyclin D3 (DCS-22)
- K0172-3 Anti-Cyclin E (HE12)
- K0173-3 Anti-Cyclin E (HE172)
- MT-19-3 Anti-Cdc2Hs (5F6)
- K0069-3 Anti-CDC6 (DCS-180)
- K0070-3 Anti-CDC7 (DCS-342)
- K0071-3 Anti-CDC25A (DCS-120)
- K0072-3 Anti-CDC25A (DCS-121)
- K0073-3 Anti-CDC25A (DCS-124)
- K0075-3 Anti-CDC25C (DCS-193)
- K0141-3 Anti-CDC27 (AF3.1)
- K0150-3 Anti-CDCP1 (CUB1)
- K0150-4 FITC labeled Anti-CDCP1 (CUB1)
- K0140-3 Anti-Cdc20 (AR12)
- K0200-3 Anti-Cdc25C (TC14)
- MK-13-3 Anti-Cdk2 (8A12)
- K0065-3 Anti-Cdk4 (DCS-156)
- K0066-3 Anti-Cdk6 (DCS-83)
- K0067-3 Anti-Cdk6 (DCS-130)
- K0068-3 Anti-Cdk7 (DCS-MO1)
- M124-3 Anti-p15<sup>INK4b</sup> (1F6)
- K0077-3 Anti-p16<sup>INK4a</sup> (DCS-50)
- K0079-3 Anti-p18<sup>INK4c</sup> (DCS-118)
- K0080-3 Anti-p19<sup>INK4d</sup> (DCS-100)
- K0081-3 Anti-p21<sup>WAF/CIP1</sup> (DCS-60)
- K0082-3 Anti-p27<sup>Kip2</sup> (DCS-72)
- K0083-3 Anti-p57<sup>Kip2</sup> (DCS-230)
- K0084-3 Anti-p14<sup>ARF</sup> (DCS-240)
- K0085-3 Anti-Cdh1 (DCS-266)
- K0086-3 Anti-Chk1 (DCS-310)
- K0087-3 Anti-Chk2 (DCS-270)
- K0088-3 Anti-Chk2 (DCS-273)
- K0094-3 Anti-E2F-4 (TFE42)
- K0095-3 Anti-DP-1 (TFD10)
- M069-3 Anti-Mcm2 (4B8)
- M038-3 Anti-Mcm3 (3A2)
- M049-3 Anti-Mcm7 (4B4)
- M050-3 Anti-RCC1 (3D11)
- MK-15-1 Anti-RB (3H9)
- M045-3 Anti-phospho RB (Ser 780) (2C4)
- 555 Anti-Phospho RB (Ser 780) (Poly)
- K0091-3 Anti-RB2 (DCS-211)
- M025-3 Anti-Phospho DNA Topoisomerase II $\alpha$  (3D4)
- M052-3 Anti-DNA Topoisomerase II  $\alpha\beta$  (AK5)
- M055-3 Anti-ORC2 (3B7)
- M057-3 Anti-GAK (1C2)
- M019-3 Anti-Nucleolin (4E2)
- PM006-3 Anti-Phospho Histone H3 (Poly)
- PM026 Anti-ATM (polyclonal)

\* CY-9050 is the product of

MBL Group  URL:<http://www.cyclex.co.jp>