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

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 α-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:

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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:

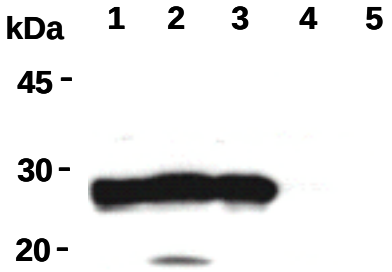
SDS-PAGE & Western Blotting

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^2 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^{INK4b} (1F6)
K0077-3	Anti-p16^{INK4a} (DCS-50)
K0079-3	Anti-p18^{INK4c} (DCS-118)
K0080-3	Anti-p19^{INK4d} (DCS-100)
K0081-3	Anti-p21$^{WAF/CIP1}$ (DCS-60)
K0082-3	Anti-p27^{Kip2} (DCS-72)
K0083-3	Anti-p57^{Kip2} (DCS-230)
K0084-3	Anti-p14^{ARF} (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 IIα (3D4)
M052-3	Anti-DNA Topoisomerase II αβ (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 CycLex URL:http://www.cyclex.co.jp