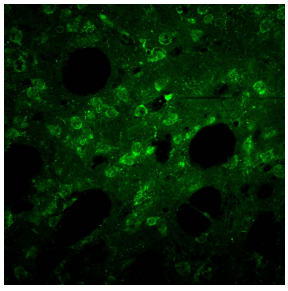
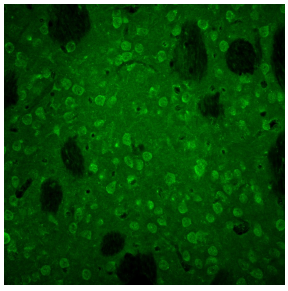
Product Data Sheet:
Dopamine Receptor D2

Cat. No:	AB-11173
Conjugate:	Unconjugated
Size:	100 ul
Clone:	POLY
Concentration:	1mg/ml
Host:	Rb
Isotype:	IgG
Reactivity:	Hu, Rt, Ms
Applications:	Elisa, Western Blot, Immunocytochemistry, Immunohistochemistry (frozen tissues) Immunocytochemistry: 1:5000 dilution (PLP fixed rat brain sections) Western Blot: whole rat brain homogenate resulted in a pair of bands being detected at ~48 and ~51 kD at 1:1000-1:10000 with ECL Detection process. In some cases, it is required a higher dilution. For IHC (F) & ICC it is important to fix the cells or tissues with the PLP fixative (Please see protocol below)
Purification:	Serum
Background:	The antiserum was raised in a rabbit which was immunized with D2 (272-282) covalently attached onto a carrier protein, and it has been characterized by immunocytochemical, western immunoblot and ELISA techniques. The antiserum has been found to be highly specific for this peptide sequence and is suitable for immunocytochemical detection of both the short and the long forms of the D2 dopamine receptor. Rehydrate the antibody with 0.1 ml of PBS which contains 10 mg/ml BSA or with additional buffer (such as 10 mg/ml BSA in PBS) for more dilute antibody.100% cross reactivity with D2 Dopamine Receptor (272-282), ~ 70% with D2S Dopamine Receptor, ~ 60% with D2L No cross reactivity with the other Dopamine Receptors.
Form:	Liquid



Working Dilutions Primary Antibody: 1:300
Tests performed by Fusco F.R., Giampà C. Neuroanatomy Lab. Fondazione S. Lucia, Roma



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Tissue processing
The animals (rats or mice) are transcardially perfused under deep anaesthesia with chloral hydrate with 60 mL of saline solution containing 0.05 mL heparin, followed by 200 mL of 4% paraformaldehyde in saline solution.
The brains are removed and postfixed overnight at +4 °C, cryoprotected in 10% sucrose and 20% glycerol in 0.1 m phosphate buffer (PB) with sodium azide 0.02% for 48 h at 4 °C. Brains were sectioned frozen on a sliding microtome at 40 lm thickness.
Single label Immunofluorescence
• Sections are rinsed for 15 min in PB containing TritonX-100 0.3%
• Sections, after a pre-incubation with the appropriate normal serum, are incubated overnight with a primary antibody at the dilution tested (1:200-1:500) in 0.1 M PB containing Triton X-100 0.3%, azide 0.02% at 4° C for three night.
• Subsequently, sections are rinsed three times for 15 min in 0.1 M PB and then incubated with Alexa Fluor 488-conjugated secondary antibody for 2 h at room temperature.
• Sections are rinsed three times for 15 min in 0.1 M PB.
• Tissue are mounted on gelatin-coated slides, coverslipped with GEL-MOUNT™ and examined under an epiillumination fluorescence.

Western Blotting Protocol

1. After SDS-PAGE (on either 4-15% gradient gels or single percentage gels, such at 12% gels) and electrophoretic transfer to PVDF membrane, block the membrane overnight with 2% normal goat serum in TBS/Tween-20 buffer.
2. Wash x 2 with TBS/Tween-20.
3. Apply the rabbit polyclonal antibody after dilution to at least 1:800 (Note: higher dilutions may be needed). Use 2% normal goat serum in TBS/Tween-20 as buffer for the primary antibody. Let the primary antibody bind for 2-4 hours.
4. Wash x 3 with TBS/Tween-20.
5. Apply affinity purified HRP-goat anti-rabbit IgG antiserum diluted 1:2500 (dilution may vary depending upon supplier) in 2% normal goat serum in TBS/Tween-20. Incubate 1-2 hours. Note: greater sensitivity may be achieved using ABC techniques.
6. Wash x 4 for 5 minute/wash with TBS/Tween-20.
7. Develop color using the enhanced DAB reaction.

Immunocytochemistry Protocol, on cellsInitially, one should perform a titration experiment to optimize the signal to noise ratio especially if peptide or protein blocking experiments are to be conducted. However, we have successfully used our monoclonal antibodies as culture supernatants, our monoclonal antibodies as ascites fluids, and our polyclonal antibodies at dilutions of at least 1:50, 1:500 and 1:1000, respectively (see specific data sheet for individual antibody). Dilute the stock antibody in PBS, pH 7.2, containing either 0.1% BSA or 1% normal goat serum.For cells cultured on slides or coverslipsWash the cells 4 times in PBS pH 7.2, rinse once quickly in distilled water, and drain well. We fix the cells with either 70% ice cold acetone or neutral buffered formalin for 10 min, air dry and store them frozen. We always allow the slides to warm to room temperature before using. If the cells were fixed with organic solvents, such as 70% acetone, 95% ethanol, or methanol, then they do not need permeabilizing. However, if fixed with formalin, formaldehyde, or glutaraldehyde, then the cells must be permeabilized before being exposed to the primary antibody. To permeabilize the cells, soak the fixed cells for 20 mins in PBS that contains 0.1% Triton X-100. Before applying the primary antibody, block non-specific binding by soaking the cells in 1% normal goat serum in PBS for 20 min. For blocked controls preincubate the diluted antibody with either 50 nmole of peptide or 50 µgm of protein per ml of diluted antibody solution for 60 minutes. Apply either the diluted primary antibody or the peptide/protein blocked antibody for 1 - 2 hours, wash 4 times in PBS, and then apply the diluted enzyme or fluorochrome labeled goat anti-mouse IgG, -mouse IgM, or -rabbit IgG second antibody for 60 min. Wash 4 times in PBS and rinse once quickly with distilled water. Mount a cover slip using a water and glycerol based mounting medium. For tissue sectionsWe routinely test two

different fixatives: 3.0% freshly depolymerized paraformaldehyde and PLP* (see below). All incubations and washing steps should be done with the tissue in constant gentle motion on an orbital shaker. We use 60 µm sections of either 3.0% paraformaldehyde or PLP* fixed whole rat brain which are cut on a freezing microtome, and washed x 4 for 15 min with PBS to remove residual fixative. Sections are then blocked and premeabilized at room temperature for 30 minutes in 1.5% normal goat serum in PBS containing 0.2% Triton X-100. Incubation with the primary antibody is performed at 4° C for 48 hours. All remaining steps are performed at room temperature. Following the incubation with the primary antibody, the sections are washed for 10 minutes in PBS to remove residual antibody before they are incubated with biotinylated goat anti-mouse IgG, -mouse IgM, or -rabbit IgG for 1 hour. After a 10 min wash in PBS, the sections are incubated with ABC reagent for 1 hour which is followed by a final wash in PBS. The sections are then transferred to 0.05 M Tris buffer, pH 7.6, and incubated for 3 minutes in 0.05% 3,3'-diaminobenzidine tetrahydrochloride and 0.005% H2O2 to visualize the reaction product. The reaction is stopped in Tris buffer and the sections transferred first to PBS then to 10 mM sodium acetate before being mounted, cleared, and coverslipped. Mounted sections are visually examined with a microscope. Control sections are incubated either with pre-immune rabbit serum or with antiserum blocked by prior incubation with the immunogen. * PLP - Periodate/Lysine/Paraformaldehyde - Ref: McLean and Nakane (1974) J Histochem Cytochem , 22 , 1077-1083 2% Paraformaldehyde, 0.1 M L-Lysine, 0.1 M Sodium meta-periodate in 0.1 M Na 2HPO4 (pH 7.4) Note : It is important to conduct initial titration experiments with the antibody on the sample. One wants good immunostaining, but it is important for blocking experiments not to have an excess of antibody because it will be impossible to block all of the antibody binding by preincubating with the peptide or protein. If one blocks 95% of the binding with the antigen, then 5% of the antibody is still available to bind. In antibody excess this will be enough to produce immunostaining of the cells or tissue.

Immunocytochemistry Protocol, on cells