

Anti-PhiYFP(d) antibody

Product	Cat.#	Lot.#	Size
Anti-PhiYFP(d) antibody	AB603	60301010805	100 μ g
	AB604	60401010805	200 μ g

Use

- Western blot
- Immunoblotting
- ICC
- ELISA

1 2 3 4 5 6 7 8 9 10 11 12 kDa 66-45-35-25-18-14-

Western blot detection of fluorescent proteins using Anti-PhiYFP(d) antibody.

1 - TagCFP; 2 - TagGFP; 3 - TagYFP; 4 - TagRFP; 5 -TurboFP602; 6 - TurboGFP; 7 - TurboYFP; 8 - TurboRFP; 9 - PS-CFP2; 10 - Dendra2; 11 - KillerRed; 12 - EGFP.

Recombinant proteins were purified from transformed *E. coli.* 25 ng of each protein were separated by SDS PAGE (14% acrylamide). The samples were boiled before loading. Antibody was used at a 1/10000 dilution. Secondary antibody: Goat anti-Rabbit HRP-conjugated IgG.

Description

Rabbit polyclonal antibody against denatured PhiYFP, PhiYFP-m, and TurboYFP.

Specificity: The antibody has been selected to recognize denatured TurboYFP, PhiYFP, and PhiYFP-m. It can also be used for recognizing non-denatured Phi-Yellow proteins, but with lesser activity than Anti-PhiYFP antibody (Cat.# AB601-AB602). The antibody shows little or no cross-reactivity with TagCFP, TagGFP, TagGFP2, TagGFP2, TagGFP2, TagGFP2, TagRFP, TurboGFP, TurboRFP, JRed, Dendra2, and KillerRed. The antibody shows cross-reactivity with TagRFP, TurboFP602, and TurboFP635.

Immunogen: Full-length recombinant denatured PhiYFP comprising 6XHis tag.

Antibody preparation: Full-length recombinant PhiYFP comprising 6XHis tag was purified from transformed *E. coli* using metal-ion affinity chromatography. Antibodies were produced in rabbits immunized with the recombinant denatured PhiYFP. Specific IgG were purified by PhiYFP affinity chromatography.

Formulation: Lyophilized from the buffer containing 0.1% mannitol, 0.1% dextran, 0.1M NaCl, 0.01M Na $_2$ HPO $_4$, and 0.01M NaBO $_4$; pH 7.4.

Reconstitution: Reconstitute with sterile water or 50% glycerol to a concentration of 1 mg/ml.

Storage: Lyophilized samples are stable for twelve months from date of receipt when stored at -20°C. The presence of silica gel drier is advisable.

Reconstituted with sterile water, antibody can be stored at +2 - +8°C for three months without detectable loss of activity.

Reconstituted with 50% glycerol, antibody can be stored at -20°C in a manual defrost freezer for six months without detectable loss of activity. Aliquot antibody upon reconstitution. Avoid repeated freeze / thaw cycles.

Recommendations for use

Anti-PhiYFP(d) antibody can be used to recognize denatured PhiYFP and PhiYFP-m proteins and their fusions. Although this antibody recognizes non-denatured PhiYFP and PhiYFP-m proteins as well, Evrogen anti-PhiYFP antibody (cat. ##AB601/ AB602) performs better for this goal.

Working concentrations:

For Western blot use at a dilution of 1:20 000;

For ELISA use at a dilution of 1:20 000 - 1:30 000;

For immunocytochemistry use at a dilution of 1:20 000.

Note: Optimal dilutions/concentrations should be determined by the end user.

Tissue (cells) fixation for immunohistochemistry: Formaldehyde (formalin, paraform) fixation is recommended. For example, tissues can be fixed in PBS containing 4% formaldehyde for 10-15 min, treated with 0.1% saponin in PBS for 10-15 min, and washed three times in PBS.

Sample preparation for Western blot: To a sample containing 10-100 ng of a target protein, add an equal volume of 2x SDS-PAGE sample buffer. Heat the sample at 95°C before loading on a gel or spotting on a membrane (for dots).