

## Anti-PHLDA3 antibody, mouse monoclonal (4B6)

71-195 100 ug

**Storage:** Ship at 4°C and store at -20°C. Do not freeze.

**Specificity:** Specific to human PHLDA3. Not tested with other species.

Immunogen: Synthetic peptide corresponding to N terminal amino acids 1-31 of human PHLDA3

Applications

1. Western blotting

2. Immunofluorescent staining (1/1,000)

**Form:** Purified monoclonal antibody (IgG) 1mg/ml in PBS, 50% glycerol, filter-sterilized **Isotype:** Mouse IgG2b kappa

Background: PHLDA3 (Pleckstrin homology-like domain family A member 3) is a small PH (Pleckstrin homology) domain-only protein (127 a.a. in human) that is highly conserved in vertebrates. Recently PHLDA3 was found to be a p53-regulated repressor of Akt (ref 2) hence it is speculated as a tumor suppressor. PHLDA3 also inhibits anchorage-independent cell growth. PHLDA3 gene is frequently lost in human lung endocrine tumors.

Data Link UniProtKB/Swiss-Prot Q9Y5J5 (PHLA3\_HUMAN)

## References This antibody has been used in Ref. 2

- Frank D et al (1999) "A novel pleckstrin homology-related gene family defined by Ip1/Tssc3, TDAG51, and Tih1: tissue-specific expression, chromosomal location, and parental imprinting" Mamm. Genome 10:1150-1159 PMID: 10594239
- 2. Kawase T *et al* (2009) "PH domain-only PHLDA3 is a novel p53-regulated repressor of Akt" *Cell* **136**: 535-550 PMID: 19203586

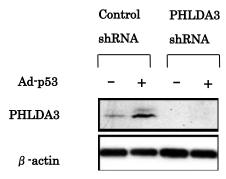


Fig.1 Western blotting was performed using the antibody 4B6 to detect PHLDA3 protein expression. PHLDA3 protein was induced by Ad-p53 in MDA-MB-468 cells (left). PHLDA3 expression was inhibited by shRNA targeting PHLDA3 (right).



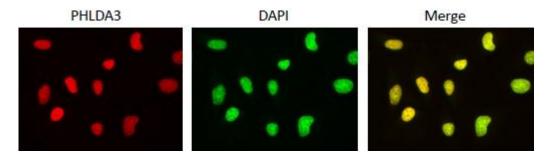


Fig.2 Immunofluorescence staining of PHLDA3 in nuclei of HeLa cells.

- 1. HeLa cells were fixed with 4% paraformaldehyde overnight, permeabilized with 0.25% Triton X-100 in PBS for 10 min.
- 2. Incubate cells with 1.5% BSA in PBS for 30 min to block non-specific binding of the antibodies. Incubate the cells with 1/1,000 diluted anti-PHLDA3 antibody in 1% BSA in PBS at  $4^{\circ}$ C overnight.
- 3. Incubate cells with a secondary antibody, goat anti-mouse IgG conjugated with Alex 488, at 1/1,000 dilution in 1% BSA for 1 hr at room temperature.
- 4. Nucleus (DNA) was stained with DAPI