PASD1

CONTACT INFORMATION:	LRF Haemato-oncology Group. University of Oxford
STATUS:	Validated
TYPE:	mouse anti human
CLONE NAME:	PASD1-2 clone 2ALCC128
PROTEIN:	PASD1 amino acids 540-773
ANTIGEN USED:	Bacterially expressed GST-PASD1 fusion protein
FUSION PARTNER:	NS0
ISOTYPE:	IgG1
SPECIES REACTIVITY:	Human
PREPARATION AND STORAGE:	Aliquot and store at 4oC. Do not freeze
APP RECOMMENDED:	IHC-paraffin, IHC-frozen, WB
APP NO TESTED:	IP, IF, Flow cytometry

DESCRIPTION

PASD1 was originally identified by our laboratory through screening the antigenic reactivity of circulating antibodies from lymphoma patients using the SEREX technique. Expression studies identified PASD1 as a novel nuclear cancer testis antigen (CTA) with normal tissue expression restricted to the testis and expression also in different cancer types, particularly lymphoma and myeloma. Some patients and lymphoma cell lines were observed to express cytoplasmic PASD1 protein. Patients were shown to mount both CD8 and CD4 T-cell responses to the PASD1 protein and a PASD1 DNA vaccine could induce immune responses in vivo. The PASD1 protein is not highly conserved in mice and the closest sequence similarity is to the CLOCK and NPAS2 proteins. Recently PASD1 has been identified as a novel regulator of the circadian clock that interacts with the CLOCK:BMAL1 complex to repress its transcriptional activation. This suggests PASD1 may molecularly link oncogenic transformation to suppression of circadian rhythms. We have generated two mouse monoclonal antibodies to PASD1. PASD1-1 (clone 2ALCC136) recognises a region common to both long and short PASD1 proteins, while PASD1-2 (clone 2ALCC128) recognises only the C-terminus of the long PASD1 protein isoform.

PUBLICATION DESCRIBING ANTIBODY CHARACTERIZATION/VALIDATION

Cooper CD, Liggins AP, Ait-Tahar K, Roncador G, Banham AH, Pulford K. PASD1, a DLBCL-associated cancer testis antigen and candidate for lymphoma immunotherapy. Leukemia. 2006 Dec;20(12):2172-4. PMID: 17024112

REFERENCES

Sahota SS, Goonewardena CM, Cooper CD, Liggins AP, Ait-Tahar K, Zojer N, Stevenson FK, Banham AH, Pulford K. PASD1 is a potential multiple myeloma-associated antigen. Blood. 2006 Dec 1;108(12):3953-5. PMID: 17114574

Ait-Tahar K, Liggins AP, Collins GP, Campbell A, Barnardo M, Lawrie C, Moir D, Hatton C, Banham AH, Pulford K. Cytolytic T-cell response to the PASD1 cancer testis antigen in patients with diffuse large B-cell lymphoma. Br J Haematol. 2009 Aug;146(4):396-407. PMID: 19552722

Ait-Tahar K, Liggins AP, Collins GP, Campbell A, Barnardo M, Cabes M, Lawrie CH, Moir D, Hatton C, Banham AH, Pulford K. CD4-positive T-helper cell responses to the PASD1 protein in patients with diffuse large B-cell lymphoma.Haematologica. 2011 Jan;96(1):78-86. PMID: 20851862

Joseph-Pietras D, Gao Y, Zojer N, Ait-Tahar K, Banham AH, Pulford K, Rice J, Savelyeva N, Sahota SS. DNA vaccines to target the cancer testis antigen PASD1 in human multiple myeloma. Leukemia. 2010 Nov;24(11):1951-9. PMID: 20861911

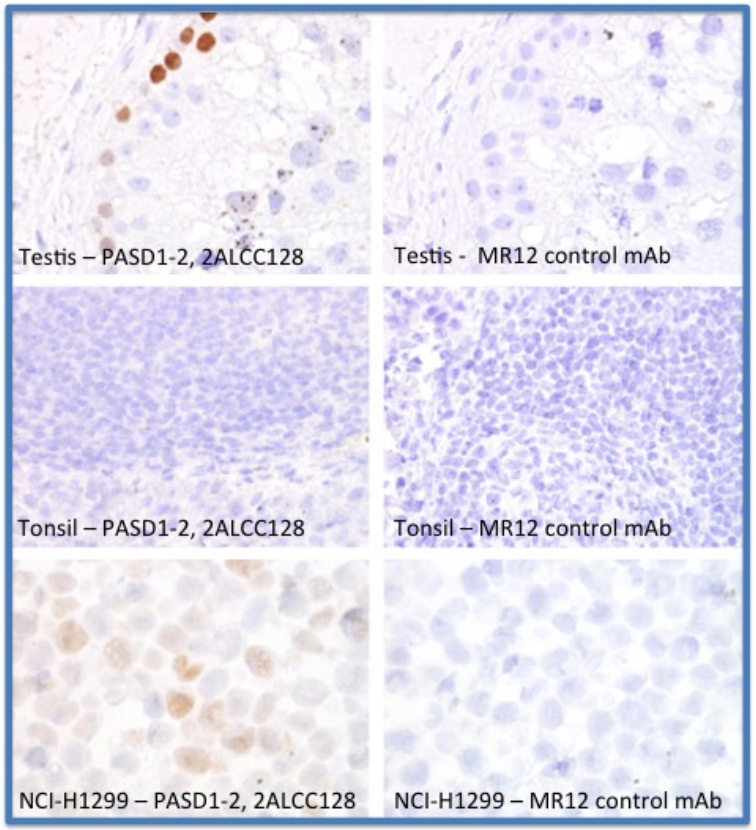
Michael AK, Harvey SL, Sammons PJ, Anderson AP, Kopalle HM, Banham AH, Partch CL. Cancer/Testis Antigen PASD1 Silences the Circadian Clock. Mol Cell. 2015 Jun 4;58(5):743-54. PMID: 25936801

Khan G, Brooks SE, Mills KI, Guinn BA. Infrequent Expression of the Cancer-Testis Antigen, PASD1, in Ovarian Cancer. Biomark Cancer. 2015 Aug 16;7:31-8. PMID: 26327782

APPLICATIONS

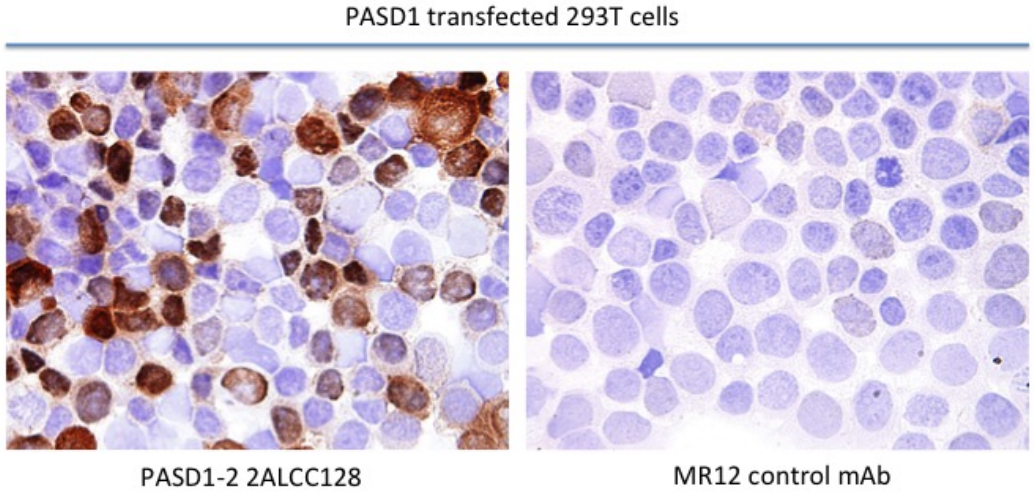
IHC Techniques	Clone	Dilution	Antibody concentration	Antigen retrieval method	Visualization kit	Positive control	Negative control	Protein localization	Positivity in other species

Frozen tissue and cytospins									
Recommended	2ALC C128	undiluted	supernatant	N/A, acetone fixation	goat anti-Mouse IgG HRP (Dako P0447 1/100)	Testis, NCI-H1299	Tonsil, MDA-MB-453	Nuclear	Human, not tested in others
Paraffin tissue									
Recommended	2ALC C128	1/15, O/N 4oC	supernatant	Tris/EDTA, presure cooker 2min	Dako Envision	Testis, NCI-H1299	Tonsil, MDA-MB-453	Nuclear	Human, not tested in others
Immunofluorescence									



IHC FFPE

Staining of formalin fixed and paraffin embedded human tissues with the PASD1-2 2ALCC128 antibody. Testis shows strong nuclear expression in a subpopulation of spermatogonia near the basal membrane in testicular tubules. Tonsil, like most normal human tissues shows no PASD1 labelling. Nuclear PASD1 protein was detectable in the lung cancer cell line NCI-H1299. The MR12 mouse monoclonal antibody was used as a negative control to demonstrate the specificity of PASD1 staining.

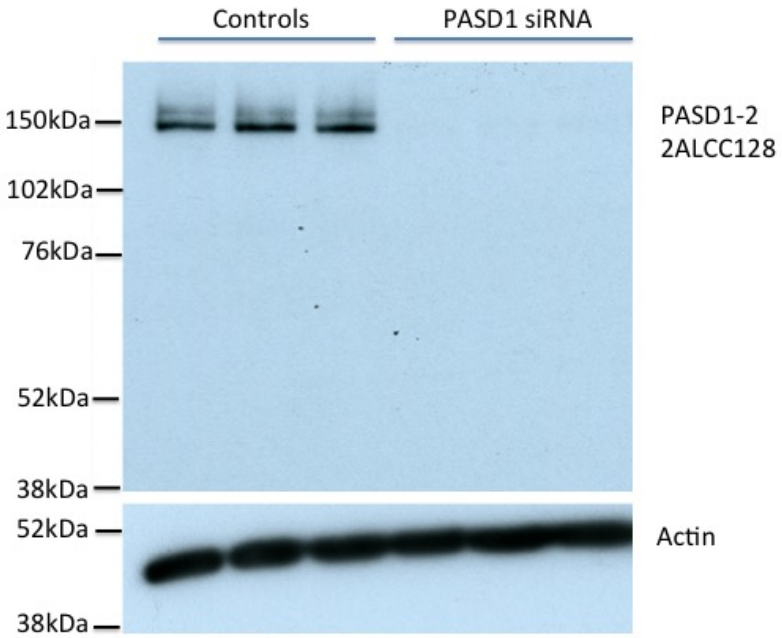


IHC frozen

293T cells were transiently transfected with cDNA expressing the long isoform of PASD1. Staining was performed with PASD1-2 clone 2ALCC128 and MR12 was used as a control antibody to detect any non-specific background. No staining of untransfected 293T cells was observed, data not shown, but note the negative cells in the PASD1-2

2ALCC128 staining which will be those that were not successfully transfected.

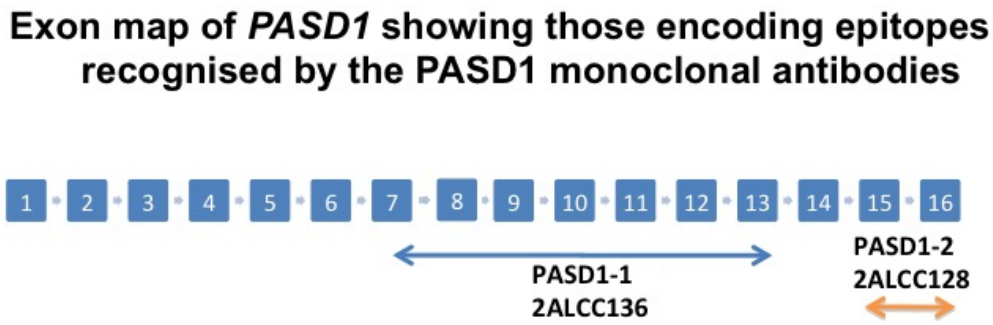
WB Techniques	Clone	Dilution	Antibody concentration	Positive control	Negative control	Expected MW	Observed Mw	Positivity in other species
Western Blotting								
Recommended	2ALCC128	1/10, O/N 4oC	supernatant	Testis, NCI-H1299	Tonsil, MDA-MB-453	~87/73kDa (isoforms 1 & 2)	~100-150kDa (often a doublet)	Human, not tested in others
Immunoprecipitation								



Western Blotting

PASD1 silencing was performed to validate the identity of the endogenous protein detected by Western Blotting in BT-20 breast cancer cells. Controls from left to right are untreated, mock siRNA, control siRNA. Three independent PASD1 siRNAs were tested. These experiments indicate that the endogenous protein detected by PASD1-2 2ALCC128 is indeed PASD1. Experiments in other cell types have given similar results. There may be some variability in the m.wt. of PASD1, 100-150kDa.

OTHERS	Title	Description
	Diagram	



PASD1 Schematic

Diagram showing the exon structure of the PASD1 gene and the regions used to generate immunogens for the production of PASD1 monoclonal antibodies.