

Vitamin D3 Receptor (D2K6W) Rabbit mAb



✓ 100 µl
(10 western blots)

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For Research Use Only. Not For Use In Diagnostic Procedures.

Entrez-Gene ID #7421
Swiss-Prot Acc. #P11473

Applications W, IP, IHC-P Endogenous	Species Cross-Reactivity* H, M, (Hm, B, Pg, Hr)	Molecular Wt. 48, 54 kDa	Isotype Rabbit IgG**
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Background: Although originally identified based on their roles in calcium and bone homeostasis, the vitamin D3 receptor (VDR/NR1H1) and its ligand 1- α , 25-dihydroxycholecalciferol [1 α , 25(OH) $_2$ D $_3$] are now recognized to exert biological effects in almost every tissue of the human body. Targets for vitamin D signaling include the central nervous system, skin, immune system, endocrine glands, kidney, and colon. At the cellular level, vitamin D signaling affects proliferation, differentiation, and apoptosis of both normal and transformed cells. Within the steroid receptor gene family, VDR belongs to the NR1H1 subfamily that also includes NR1H2/PXR and NR1H3/CAR. The human VDR gene is composed of 11 exons that encode six domains (A-F) of the full length VDR protein, which includes an N-terminal dual zinc finger DNA binding domain, a C-terminal ligand-binding activity domain, and an extensive unstructured region that links the two functional domains together (1). Upon 1 α , 25(OH) $_2$ D $_3$ binding to the hormone ligand-binding domain, VDR is stabilized by the phosphorylation of Ser51 in the DNA-binding domain by PKC (2), and Ser208 in the hinge region by casein kinase II (3). VDR associates with the retinoic acid receptor (RXR) through dimerization domains. The 1 α , 25(OH) $_2$ D $_3$ -VDR-RXR complex binds to the vitamin D response elements (VDREs) in the promoters of target genes through the DNA-binding domain. Ligand-induced conformation changes in VDR results in the dissociation of the co-repressor, silencing-mediator for retinoid and thyroid hormone receptors (SMRT), and allows interaction of the VDR activation function (AF2) transactivation domain with transcriptional coactivators (1).

Studies have shown that variable VDR expression is associated with different forms or stages of cancer and likely results from tissue-type variation in 1 α , 25(OH) $_2$ D $_3$ signaling. In the case of colon cancer, research indicates that VDR expression is relatively higher in hyperplastic colon polyps and during early tumorigenesis but diminishes in later stage, poorly differentiated tumors. Multiple studies suggest that 1 α , 25(OH) $_2$ D $_3$ may be an attractive target for development as a therapeutic anticancer agent (4,5).

Specificity/Sensitivity: Vitamin D3 Receptor (D2K6W) Rabbit mAb recognizes endogenous levels of total vitamin D3 receptor protein. This antibody does not cross-react with vitamin D3 receptor-like proteins. Based upon sequence alignment, this antibody is predicted to react with both VDRB1 and VDRB2 isoforms.

Source/Purification: Monoclonal antibody is produced by immunizing animals with a synthetic peptide corresponding to residues near the amino terminus of human vitamin D receptor isoform A protein.

Background References:

- (1) Haussler, M.R. et al. (1998) *J Bone Miner Res* 13, 325-49.
- (2) Hsieh, J.C. et al. (1991) *Proc Natl Acad Sci USA* 88, 9315-9.
- (3) Jurutka, P.W. et al. (1993) *J Biol Chem* 268, 6791-9.
- (4) Matusiak, D. et al. (2005) *Cancer Epidemiol Biomarkers Prev* 14, 2370-6.
- (5) Deeb, K.K. et al. (2007) *Nat Rev Cancer* 7, 684-700.

Storage: Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA, 50% glycerol and less than 0.02% sodium azide. Store at -20°C. Do not aliquot the antibody.

***Species cross-reactivity is determined by western blot.**

****Anti-rabbit secondary antibodies must be used to detect this antibody.**

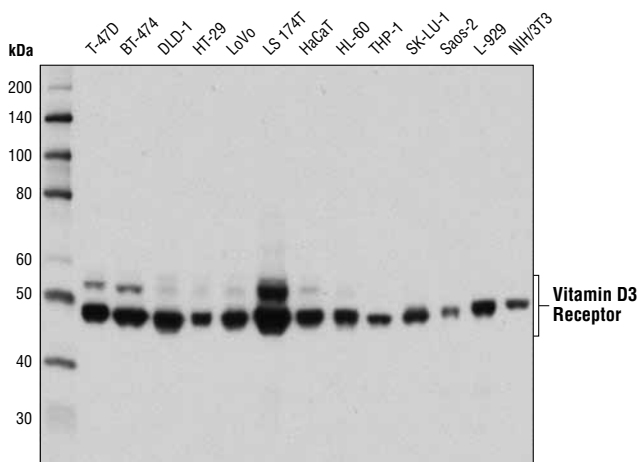
Recommended Antibody Dilutions:

Western blotting	1:1000
Immunoprecipitation	1:100
Immunohistochemistry (Paraffin)	1:200†
Unmasking buffer:	Citrate
Antibody diluent:	SignalStain® Antibody Diluent #8112
Detection reagent:	SignalStain® Boost (HRP, Rabbit) #8114

†Optimal IHC dilutions determined using SignalStain® Boost IHC Detection Reagent.

For product specific protocols please see the web page for this product at www.cellsignal.com.

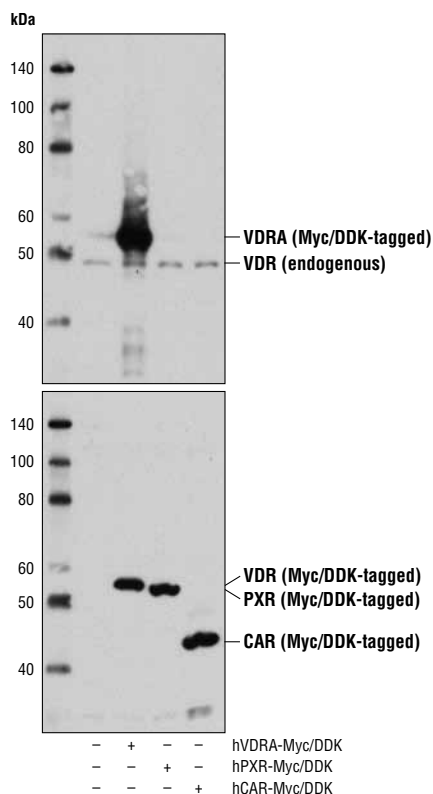
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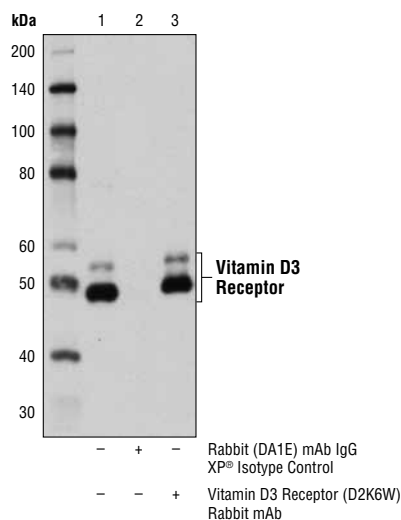
Western blot analysis of extracts from various cell lines using Vitamin D3 Receptor (D2K6W) Rabbit mAb.

IMPORTANT: For western blots, incubate membrane with diluted antibody in 5% w/v nonfat dry milk, 1X TBS, 0.1% Tween-20 at 4°C with gentle shaking, overnight.

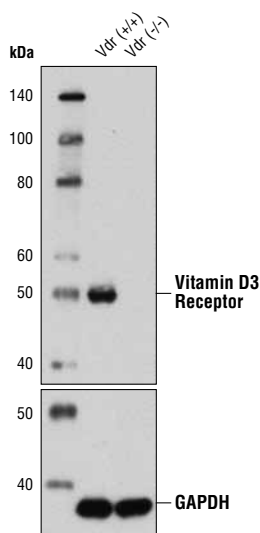
Applications Key: W—Western IP—Immunoprecipitation IHC—Immunohistochemistry ChIP—Chromatin Immunoprecipitation IF—Immunofluorescence F—Flow cytometry E-P—ELISA-Peptide
Species Cross-Reactivity Key: H—human M—mouse R—rat Hm—hamster Mk—monkey Mi—mink C—chicken Dm—D. melanogaster X—Xenopus Z—zebrafish B—bovine
Dg—dog Pg—pig Sc—S. cerevisiae Ce—C. elegans Hr—horse All—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.



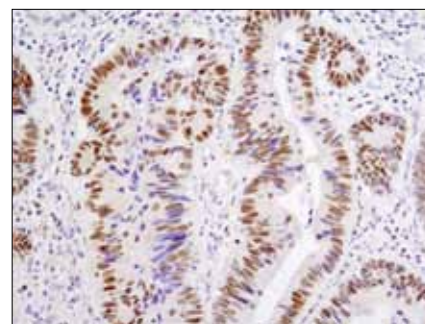
Western blot analysis of extracts from 293T cells, mock transfected (-) or transfected with constructs expressing Myc/DDK-tagged full-length human vitamin D receptor isoform A (hVDRA-Myc/DDK; +), full-length human pregnane X receptor (hPXR-Myc/DDK; +), and full-length human constitutive androstane receptor (hCAR-Myc/DDK; +), using Vitamin D3 Receptor (D2K6W) Rabbit mAb (upper) or DYKDDDDK Tag Antibody #2368 (lower).



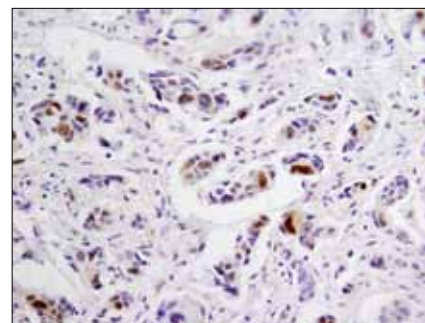
Immunoprecipitation of vitamin D3 receptor from T-47D cell extracts, using Rabbit (DA1E) mAb IgG XP® Isotype Control #3900 (lane 2) or Vitamin D3 Receptor (D2K6W) Rabbit mAb (lane 3). Lane 1 is 10% input. Western blot analysis was performed using Vitamin D3 Receptor (D2K6W) Rabbit mAb.



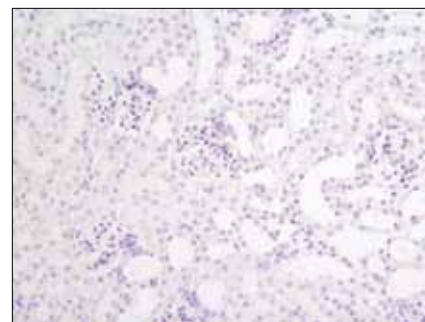
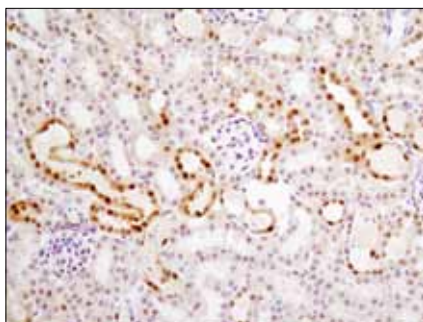
Western blot analysis of kidney tissue extracts from Vdr (+/+) and Vdr (-/-) mice using Vitamin D3 Receptor (D2K6W) Rabbit mAb (upper) and GAPDH (D16H11) XP® Rabbit mAb #5174 (lower). (Vdr (+/+) and Vdr (-/-) kidneys were kindly provided by Dr. Marie Demay, Massachusetts General Hospital).



Immunohistochemical analysis of paraffin-embedded human colon carcinoma using Vitamin D3 Receptor (D2K6W) Rabbit mAb.



Immunohistochemical analysis of paraffin-embedded human breast carcinoma using Vitamin D3 Receptor (D2K6W) Rabbit mAb.



Immunohistochemical analysis of paraffin-embedded mouse kidney, Vdr (+/+) (left) or Vdr (-/-) (right), using Vitamin D3 Receptor (D2K6W) Rabbit mAb (Tissues courtesy of Dr. Marie Demay, Massachusetts General Hospital).