Androgen Receptor (D6F11) XP® Rabbit mAb

Background: Androgen receptor (AR), a zinc finger transcription factor belonging to the nuclear receptor superfamily, is activated by phosphorylation and dimerization upon ligand binding (1). This promotes nuclear localization and binding of AR to androgen response elements in the androgen target genes. AR plays a crucial role in several stages of male development and the progression of prostate cancer (2,3).

Specificity/Sensitivity: Androgen Receptor (D6F11) XP® Rabbit mAb detects endogenous levels of total androgen receptor protein.

Source/Purification: Monoclonal antibody is produced by immunizing animals with recombinant protein corresponding to residues near the amino terminal region of human androgen receptor protein.

Background References:

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 1:2000
Immunoprecipitation 1:50
Immunohistochemistry (Paraffin) 1:200-1:800
Optimal IHC dilutions determined using SignalStain® Boost IHC Detection Reagent.
Unmasking buffer: Citrate Antibody Diluent: SignalStain® Antibody Diluent #8112 Detection reagent: SignalStain® Boost (HRP, Rabbit) #8114
Chromatin IP/Chromatin IP-seq 1:100
Optimal ChIP/ChIP-seq conditions: 5 µl of antibody & 10 µg of chromatin (4 x 10^6 cells) per IP. Antibody validated using SimpleChIP® Enzymatic ChIP Kits.
Immunofluorescence 1:600-1:1200
Flow Cytometry 1:400-1:800

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

Please visit www.cellsignal.com for a complete listing of recommended companion products.

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.

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Immunohistochemical analysis of paraffin-embedded LNCaP (AR+, left) and DU145 (AR-, right) using Androgen Receptor (D6F11) XP® Rabbit mAb.

Immunohistochemical analysis of paraffin-embedded human prostate carcinoma using Androgen Receptor (D6F11) XP® Rabbit mAb.

Flow cytometric analysis of DU-145 cells (blue) and LNCaP cells (green) using Androgen Receptor (D6F11) XP® Rabbit mAb (solid lines) or a concentration matched Rabbit (DA1E) mAb IgG XP® Isotype Control #3900 (dashed lines). Anti-rabbit IgG (H+L), F(ab')2 Fragment (Alexa Fluor® 488 Conjugate) #4412 was used as a secondary antibody.

Chromatin immunoprecipitations were performed with cross-linked chromatin from LNCaP cells grown in phenol red free medium and 5% charcoal stripped FBS for 3 d then treated with dihydrotestosterone (DHT, 10 nM) for 4 hours and either Androgen Receptor (D6F11) XP® Rabbit mAb or Normal Rabbit IgG #2729 using SimpleChIP® Plus Enzymatic Chromatin IP Kit (Magnetic Beads) #9005. The enriched DNA was quantified by real-time PCR using SimpleChIP® Human KLK2 Intron 1 Primers #62086, SimpleChIP® Human KLK3 Promoter Primers #32784, and SimpleChIP® Human α Satellite Repeat Primers #4486. The amount of immunoprecipitated DNA in each sample is represented as signal relative to the total amount of input chromatin, which is equivalent to one.

Chromatin immunoprecipitations were performed with cross-linked chromatin from LNCaP cells grown in phenol red free medium and 5% charcoal stripped FBS for 3 d then treated with dihydrotestosterone (DHT, 10 nM) for 4 hours and Androgen Receptor (D6F11) XP® Rabbit mAb, using SimpleChIP® Plus Enzymatic Chromatin IP Kit (Magnetic Beads) #9005. DNA Libraries were prepared using SimpleChIP® ChIP-seq DNA Library Prep Kit for Illumina® #56795. The figure shows binding across chromosome 19 (upper), including KLK2 (lower), a known target gene of Androgen Receptor (see additional figure-containing ChIP-qPCR data).