Store at -20°C

Androgen Receptor Sampler Kit

#61949

1 Kit
(3 x 20 µl)

New 04/19

Support: +1-978-867-2388 (U.S.)
www.cellsignal.com/support

Orders: 877-616-2355 (U.S.)
orders@cellsignal.com

Entrez-Gene ID #367
UniProt ID #P10275

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

Products Included	Product #	Quantity	Mol. Wt.	Isotype
Androgen Receptor (E3S4N) Rabbit mAb (C-Terminal Antigen)	70317	20 µl	110 kDa	Rabbit IgG
Androgen Receptor (D6F11) XP(R) Rabbit mAb	5153	20 µl	110 kDa	Rabbit IgG
Androgen Receptor (AR-V7 Specific) (E3O8L) Rabbit mAb	19672	20 µl	80 kDa	Rabbit IgG
Anti-Rabbit IgG, HRP-linked Antibody	7074	100 µl		Goat

See www.cellsignal.com for individual component applications, species cross-reactivity, dilutions and additional application protocols.

Description: The Androgen Receptor Antibody Sampler Kit provides an economical means of detecting full-length Androgen Receptor and AR-V7 isoforms. The kit includes enough antibody to perform two western blot experiments with each primary antibody.

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 androgen target genes. Research studies have shown that AR plays a crucial role in several stages of male development and the progression of prostate cancer (2,3).

The AR3 or AR-V7 isoform, which lacks the typical ligand binding domain, is created through the alternative splicing of cryptic exons (4-5). AR-V7 is frequently expressed in castration-resistant prostate cancer (CRPC) and while dependent on the activity of the full-length androgen receptor (AR-FL), AR-V7 can activate a completely distinct transcriptional program (6-8). While enzalutamide and abiraterone have been beneficial in treating CRPC through the ligand binding domain of AR-FL, resistance in patients has been shown to be associated with AR-V7 detection in circulating tumor cells (9-12). Studies probing into mechanisms of overcoming this resistance are currently being explored and may help in stratifying patient populations for more personalized therapies (13-15).

Specificity/Sensitivity: Each antibody in the Androgen Receptor Antibody Sampler kit detects endogenous levels of its target protein. Androgen Receptor (D6F11) XP® Rabbit mAb detects both full-length AR and the AR-V7 isoform. Androgen Receptor (E3S4N) Rabbit mAb (Carboxy-terminal Antigen) detects only full-length AR. Androgen Receptor (AR-V7 Specific) (E3O8L) Rabbit mAb only detects the AR-V7 isoform.

Source/Purification: Monoclonal antibodies are produced by immunizing rabbits with recombinant protein corresponding to residues near the amino terminal region of human androgen receptor protein, and with synthetic peptides corresponding to residues surrounding Val662 of human androgen receptor protein and Leu639 of human androgen receptor (V7 isoform) protein.

Background References:

(1) Li, J. and Al-Azzawi, F. (2009) *Maturitas* 63, 142-8.
(2) Avila, D.M. et al. *J Steroid Biochem Mol Biol* 76, 135-42.
(3) Montgomery, J.S. et al. (2001) *J Pathol* 195, 138-46.
(4) Hu, R. et al. (2009) *Cancer Res* 69, 16-22.
(5) Guo, Z. et al. (2009) *Cancer Res* 69, 2305-13.
(6) Watson, P.A. et al. (2010) *Proc Natl Acad Sci U S A* 107, 16759-65.
(7) Sun, S. et al. (2010) *J Clin Invest* 120, 2715-30.
(8) Hu, R. et al. (2012) *Cancer Res* 72, 3457-62.
(9) Scher, H.I. et al. (2012) *N Engl J Med* 367, 1187-97.
(10) de Bono, J.S. et al. (2011) *N Engl J Med* 364, 1995-2005.
(11) Ryan, C.J. et al. (2013) *N Engl J Med* 368, 138-48.
(12) Antonarakis, E.S. et al. (2014) *N Engl J Med* 371, 1028-38.
(13) Liu, C. et al. (2014) *Clin Cancer Res* 20, 3198-3210.
(14) Sarwar, M. et al. (2016) *Oncotarget* 7, 63065-63081.
(15) Ku, S.Y. et al. (2017) *Science* 355, 78-83.

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Applications: **W**—Western **IP**—Immunoprecipitation **IHC**—Immunohistochemistry **ChIP**—Chromatin Immunoprecipitation **IF**—Immunofluorescence **F**—Flow cytometry **E-P**—ELISA-Peptide **Species Cross-Reactivity:** **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 Immunoblotting Protocol

For western blots, incubate membrane with diluted primary antibody in either 5% w/v BSA or nonfat dry milk, 1X TBS, 0.1% Tween® 20 at 4°C with gentle shaking, overnight.
NOTE: Please refer to primary antibody datasheet or product webpage for recommended primary antibody dilution buffer and recommended antibody dilution.

A. Solutions and Reagents

NOTE: Prepare solutions with reverse osmosis deionized (RODI) or equivalent grade water.

1. **20X Phosphate Buffered Saline (PBS):** (#9808) To prepare 1 L 1X PBS: add 50 ml 20X PBS to 950 ml dH_2O, mix.
2. **10X Tris Buffered Saline (TBS):** (#12498) To prepare 1 L 1X TBS: add 100 ml 10X to 900 ml dH_2O, mix.
3. **1X SDS Sample Buffer:** Blue Loading Pack (#7722) or Red Loading Pack (#7723) Prepare fresh 3X reducing loading buffer by adding 1/10 volume 30X DTT to 1 volume of 3X SDS loading buffer. Dilute to 1X with dH_2O.
4. **10X Tris-Glycine SDS Running Buffer:** (#4050) To prepare 1 L 1X running buffer: add 100 ml 10X running buffer to 900 ml dH_2O, mix.
5. **10X Tris-Glycine Transfer Buffer:** (#12539) To prepare 1 L 1X transfer buffer: add 100 ml 10X transfer buffer to 200 ml methanol + 700 ml dH_2O, mix.
6. **10X Tris Buffered Saline with Tween® 20 (TBST):** (#9997) To prepare 1 L 1X TBST: add 100 ml 10X TBST to 900 ml dH_2O, mix.
7. **Nonfat Dry Milk:** (#9999)
8. **Blocking Buffer:** 1X TBST with 5% w/v nonfat dry milk; for 150 ml, add 7.5 g nonfat dry milk to 150 ml 1X TBST and mix well.
9. **Wash Buffer:** (#9997) 1X TBST
10. **Bovine Serum Albumin (BSA):** (#9998)
11. **Primary Antibody Dilution Buffer:** 1X TBST with 5% BSA or 5% nonfat dry milk as indicated on primary antibody datasheet; for 20 ml, add 1.0 g BSA or nonfat dry milk to 20 ml 1X TBST and mix well.
12. **Biotinylated Protein Ladder Detection Pack:** (#7727)
13. **Prestained Protein Marker, Broad Range (Premixed Format):** (#7720)
14. **Blotting Membrane and Paper:** (#12369) This protocol has been optimized for nitrocellulose membranes. Pore size 0.2 µm is generally recommended.
15. **Secondary Antibody Conjugated to HRP:** anti-rabbit (#7074); anti-mouse (#7076)
16. **Detection Reagent:** LumiGLO® chemiluminescent reagent and peroxide (#7003) or SignalFire™ ECL Reagent (#6883)

B. Protein Blotting

A general protocol for sample preparation.

1. Treat cells by adding fresh media containing regulator for desired time.
2. Aspirate media from cultures; wash cells with 1X PBS; aspirate.
3. Lyse cells by adding 1X SDS sample buffer (100 µl per well of 6-well plate or 500 µl for a 10 cm diameter plate). Immediately scrape the cells off the plate and transfer the extract to a microcentrifuge tube. Keep on ice.
4. Sonicate for 10–15 sec to complete cell lysis and shear DNA (to reduce sample viscosity).
5. Heat a 20 µl sample to 95–100°C for 5 min; cool on ice.
6. Microcentrifuge for 5 min.
7. Load 20 µl onto SDS-PAGE gel (10 cm x 10 cm). **NOTE:** Loading of prestained molecular weight markers (#7720, 10 µl/lane) to verify electrotransfer and biotinylated protein ladder (#7727, 10 µl/lane) to determine molecular weights are recommended.
8. Electrotransfer to nitrocellulose membrane (#12369).

C. Membrane Blocking and Antibody Incubations

NOTE: Volumes are for 10 cm x 10 cm (100 cm^2) of membrane; for different sized membranes, adjust volumes accordingly.

I. Membrane Blocking

1. (Optional) After transfer, wash nitrocellulose membrane with 25 ml TBS for 5 min at room temperature.
2. Incubate membrane in 25 ml of blocking buffer for 1 hr at room temperature.
3. Wash three times for 5 min each with 15 ml of TBST.

II. Primary Antibody Incubation

1. Incubate membrane and primary antibody (at the appropriate dilution and diluent as recommended in the product datasheet) in 10 ml primary antibody dilution buffer with gentle agitation overnight at 4°C.
2. Wash three times for 5 min each with 15 ml of TBST.
3. Incubate membrane with the species appropriate HRP-conjugated secondary antibody (#7074 or #7076 at 1:2000) and anti-biotin, HRP-linked Antibody (#7075 at 1:1000–1:3000) to detect biotinylated protein markers in 10 ml of blocking buffer with gentle agitation for 1 hr at room temperature.
4. Wash three times for 5 min each with 15 ml of TBST.
5. Proceed with detection (Section D).

D. Detection of Proteins

1. Incubate membrane with 10 ml LumiGLO® (0.5 ml 20X LumiGLO® #7003, 0.5 ml 20X peroxide, and 9.0 ml purified water) or 10 ml SignalFire™ #6883 (5 ml Reagent A, 5 ml Reagent B) with gentle agitation for 1 min at room temperature.
2. Drain membrane of excess developing solution (do not let dry), wrap in plastic wrap and expose to x-ray film. An initial 10 sec exposure should indicate the proper exposure time. **NOTE:** Due to the kinetics of the detection reaction, signal is most intense immediately following incubation and declines over the following 2 hr.