

NR Sandwich AR ELISA

Catalog Nos. 49196 & 49696

(version B1)

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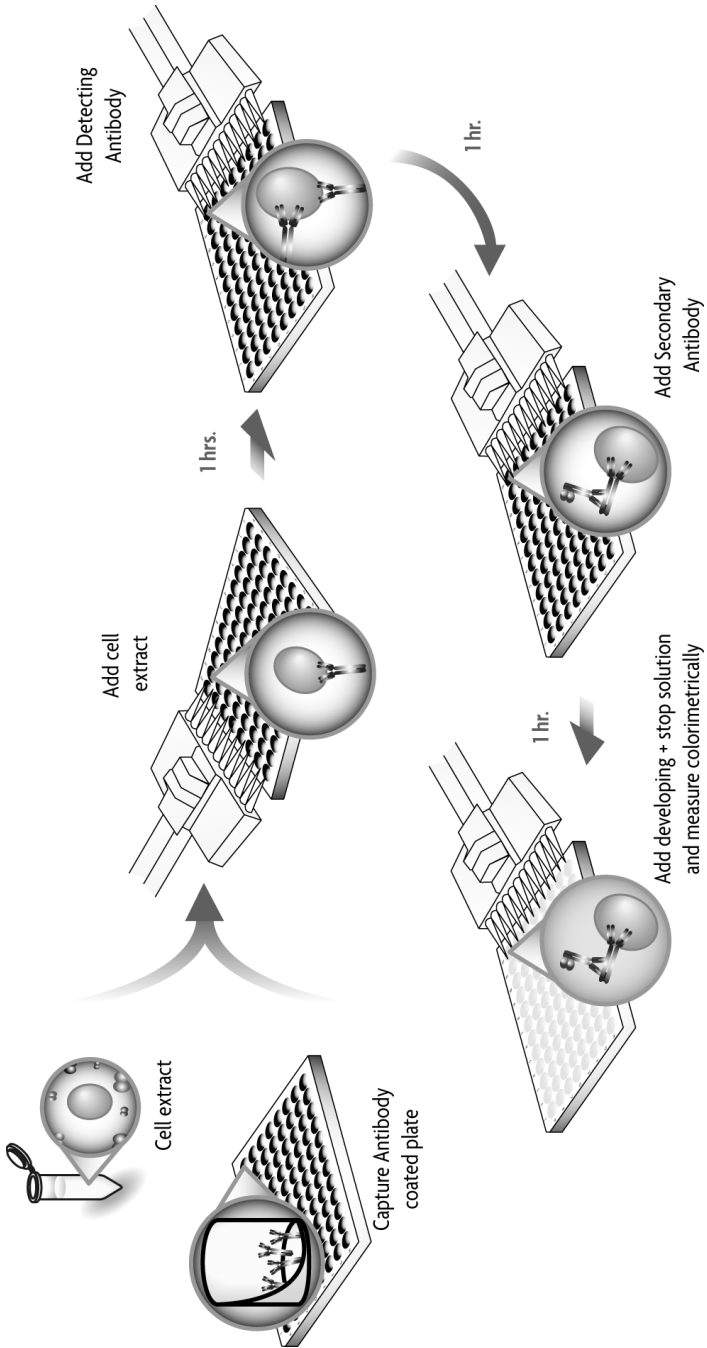
Overview

Androgen Receptor (AR) belongs to the nuclear receptor (NR) superfamily of structurally related ligand-inducible transcription factors¹. NRs act in combination with other transcription factors to regulate the expression of gene networks involved in cell growth and development, apoptosis, homeostasis, inflammation, lipid metabolism, the reproductive cycle and other fundamental biological processes. AR expression plays an important role in the proliferation of human prostate cancer and also confers a better prognosis in breast cancer^{2,3}. Because of AR's critical role in cell biology, it is important to measure the total amounts of AR contained in different cell types and tissues. Traditional methods for monitoring AR protein levels, such as Western blotting, EMSA, immunohistochemistry (IHC) and reporter gene assays, are time consuming and not suitable to high-throughput applications.

With its NR Sandwich ELISAs, Active Motif is introducing the first ELISA-based kits to detect total amounts of nuclear receptor proteins. The NR Sandwich AR Kit simplifies the measurement of AR contained in cell and tissue samples by using the “Sandwich ELISA” method for detecting a protein. This method uses two antibodies that each recognize a distinct epitope on the protein of interest. The kit provides an ELISA plate that is coated with the first antibody, called the Capture Antibody, which is used to capture the protein from the sample. The second antibody, called the Detecting Antibody, is used to detect the protein bound by the Capture Antibody. An HRP-conjugated Secondary Antibody is then used to quantitate the amount of bound Detecting Antibody. Subsequent incubation with developing solution provides an easily quantified colorimetric readout. NR Sandwich AR is available in two sizes:

| product | format | catalog no. |
|----------------|--------------------|--------------------|
| NR Sandwich AR | 1 x 96-well plate | 49196 |
| | 5 x 96-well plates | 49696 |

Flow Chart of Process



Introduction

Androgen Receptor

Androgens play an important role in the development, maintenance and regulation of the reproductive physiology of adult males, including maintenance of the prostate. Androgens exert their effects via the intracellular androgen receptor (AR), which belongs to the nuclear receptor (NR) superfamily of ligand-regulated transcription factors. Like other members of the NR family, AR consists of an N-terminal modulating domain, a central DNA binding domain, a hinge region and a C-terminal ligand-binding domain⁴. AR expression plays a critical role in the proliferation of human prostate cancer and also confers a better prognosis in breast cancer^{2,3}.

Primary prostatic cancers are largely dependent on androgens for growth and survival. Patients with prostate cancer are usually subjected to hormonal therapy by either androgen deprivation and/or blockade of AR with anti-androgens. These treatments are beneficial in the early stages of cancer but eventually lead to relapse of androgen-insensitive cancers. Paradoxically, many hormone-insensitive prostate cancers are found to be positive for AR, suggesting AR may contribute to the progression of androgen-independent prostate cancer. Mutations in the AR gene are rare in hormone-refractory prostate cancer and do not play an important role. Instead, the amplification and consequent overexpression of the wild-type AR gene appears to be the most common alteration involving AR in hormone-refractory prostate cancer⁵⁻⁸. AR gene amplification has been reported in about 30% of hormone-refractory tumors^{9,10}, indicating AR's regulatory role in tumor growth from patients with clinically defined androgen-independent prostate cancer¹¹. The expression of AR is reportedly inversely correlated with histologic grade: well-differentiated prostate tumors show higher expression than the poorly differentiated tumors. In prostate cancer, AR has been proposed as a marker of hormone-responsiveness.

Androgens are also of biological and clinical importance for the growth and development of breast cancer in women. ARs are present in about 60% of breast carcinomas and about 18% of AR-positive cells lack ER and PR¹². AR has also been shown to be a predictor of tumor differentiation¹³.

Traditional Nuclear Receptor Assays

To date, several methods are widely used to measure AR expression, either directly or indirectly:

1. Cellular levels of AR protein can be determined by Western blot by using antibodies specific for AR protein. This method is time consuming (up to 2 days once the nuclear extracts are prepared), and is not suitable for processing large numbers of samples.
2. The DNA-binding capacity of AR can be assayed by gel retardation, also called electrophoretic mobility shift assay (EMSA). In this method, nuclear extracts are incubated with a radioactive double-stranded oligonucleotide probe containing the consensus sequence for AR binding. If AR is active in the nuclear extract, it will bind to the probe. Samples are then resolved by electrophoresis on a native polyacrylamide gel, followed by autoradiography.

This method is sensitive, but like the previous procedure, it is time consuming (multiple days of gel exposure may be required to achieve sufficient sensitivity) and it cannot be applied to high-throughput screening. Gelshift assays also require special precautions and equipment for handling radioactivity.

3. Immunohistochemistry is also commonly performed to analyze the AR content of tissue samples. Although this method is highly sensitive and can reveal the subcellular distribution of AR protein, it is technically demanding, requires specialized equipment, and is not suitable for the analysis of a large number of samples
4. Another method used to assay AR activation is based on reporter genes, typically luciferase or β -galactosidase, placed under the control of a promoter containing an AR consensus binding site. The promoter can be artificial, made of a GC box and a TATA box, or natural. However, the procedure is limited by the following issues: (i) reporter gene assays have to be repeated several times to obtain statistically reliable data; and (ii) reporter gene assays are sensitive to confounding factors that may influence the expression level of the reporter gene; therefore, assays have to be carefully standardized.

NR Sandwich AR ELISAs

AR-regulated genes are involved in a variety of cellular pathways that are currently being deciphered by academic and pharmaceutical laboratories for new target discovery. However, there is a lack of standardized assays that measure cellular levels of AR.

To overcome this, Active Motif has introduced the NR Sandwich AR to monitor the expression levels of AR in cell and tissue samples. The NR Sandwich AR ELISA Kit uses the “Sandwich ELISA” method for detecting a protein. This method uses two antibodies that each recognize a distinct epitope on the protein of interest. The kit provides an ELISA plate that is coated with the first antibody, called the Capture Antibody, which is used to capture the protein from the sample. The second antibody, called the Detecting Antibody, is used to detect the protein bound by the Capture Antibody. An HRP-conjugated Secondary Antibody is then used to quantitate the amount of bound Detecting Antibody. Subsequent incubation with developing solution provides an easily quantified colorimetric readout. Once the samples are prepared, this assay is completed in less than 4 hours. As this assay is performed in 96-well plates, a large number of samples can be handled simultaneously, enabling high-throughput automation. This assay is specific for AR and can be used to detect AR in as little as 0.6 μ g of nuclear extract from LNCaP cells.

The NR Sandwich AR has many applications including the study of AR transcriptional activity regulation and protein structure/function studies of AR in cancer development and progression.

Kit Performance and Benefits

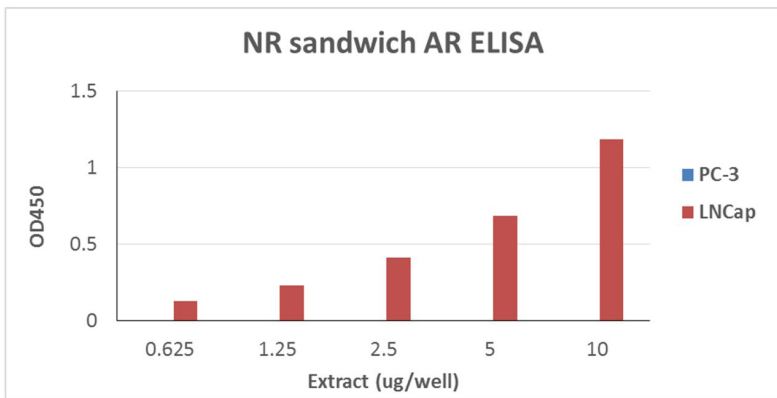
The NR Sandwich AR Kit is for research use only. Not for use in diagnostic procedures.

Detection limit: > 0.6 µg nuclear extract/well.

Range of detection: NR Sandwich AR provides quantitative results from 0.6 to 10 µg of nuclear extract/well (see graph below).

Cross-reactivity: NR Sandwich AR detects AR from human origin. This assay is not recommended for use with samples from mouse origin. Reactivity with other species has not been determined.

Assay time: < 4 hours.



Monitoring protein expression levels of AR using NR Sandwich. Different amounts of nuclear extracts from two human prostate cancer cell lines: LNCaP and PC-3 were analyzed for levels of AR protein using the NR Sandwich AR Kit. This data is provided for demonstration purposes only.

NR Sandwich AR ELISA Kit Components and Storage

Except for the nuclear extract that must be kept at -80°C, kit components can be stored at -20°C prior to first use. Then, we recommend storing each component at the temperature indicated in the table below.

| Reagents | Quantity (1 plate / 5 plates) | Storage / Stability |
|---|----------------------------------|---------------------|
| AR detecting antibody | 12 µl / 60 µl | 4°C for 6 months |
| Anti-rabbit HRP-conjugated antibody (0.25 µg/µl) | 12 µl / 60 µl | 4°C for 6 months |
| LNCaP nuclear extract (5 µg/µl) | 40 µl / 200 µl | -80°C for 6 months |
| Diluent Buffer | 22 ml / 110 ml | -20°C for 6 months |
| 10X Wash Buffer AM1 | 22 ml / 110 ml | 4°C for 6 months |
| Developing Solution | 11 ml / 55 ml | 4°C for 6 months |
| Stop Solution | 11 ml / 55 ml | 4°C for 6 months |
| 96-well assay plate | 1 / 5 | 4°C for 6 months |
| Plate sealer | 1 / 5 | Room temperature |

Additional Materials Required

- Multi-channel pipettor
- Multi-channel pipettor reservoirs
- Rocking platform
- Microplate spectrophotometer capable of reading at 450 nm (655 nm as optional reference wavelength).

For Nuclear Extract preparation

- Hypotonic Buffer
- Phosphatase Inhibitor Buffer
- 10X PBS
- Detergent (NP-40)
- Lysis Buffer

Protocols

Buffer Preparation and Recommendations

Preparation of 1X Wash Buffer

Prepare the amount of 1X Wash Buffer required for the assay as follows: For every 10 ml of 1X Wash Buffer required, dilute 1 ml 10X Wash Buffer AMI with 9 ml distilled water (see the Quick Chart for Preparing Buffers and Controls in this section). Mix gently to avoid foaming. The 1X Wash Buffer may be stored at 4°C for one week. The Tween 20 contained in the 10X Wash Buffer AMI may form clumps, therefore homogenize the buffer by vortexing for 2 minutes prior to use.

Preparation of the Antibody Binding Buffers

Dilute the AR detecting antibody to 1:1,000 and anti-rabbit HRP-conjugated secondary antibody to 1:1,000 with the Diluent Buffer (see the Quick Chart in this section). Use 50 µl of diluted antibody per well. Depending on the particular assay, the signal:noise ratio may be optimized by using higher dilutions of both antibodies. This may decrease the sensitivity of the assay.

Developing Solution

The Developing Solution must be warmed to room temperature before use. This solution is light sensitive, therefore, we recommend avoiding direct exposure to intense light during storage. The Developing Solution may develop a yellow hue over time. This does not affect product performance. A blue color present in the solution indicates that it has been contaminated and must be discarded. Prior to use, transfer the amount of Developing Solution required for the assay into a secondary container (see the Quick Chart for Preparing Buffers in this section), avoid direct exposure to intense light and leave at room temperature for at least 1 hour. After use, discard any remaining solution that was transferred into the secondary container.

Stop Solution

Prior to use, transfer the amount of Stop Solution required for the assay into a secondary container (see the Quick Chart for Preparing Buffers and Controls in this section). After use, discard remaining Stop Solution.

WARNING: The Stop Solution is corrosive. Wear personal protective equipment when handling, *i.e.* safety glasses, gloves and labcoat.

Nuclear Extract

The LNCaP nuclear extract is provided as a positive control to ensure that the kit reagents are functional. Sufficient extract is provided for 40 reactions. This extract is optimized to give a strong signal when used at 5 µg/well. We recommend aliquoting the extract in 5 µl fractions and storing at -80°C. Avoid multiple freeze/thaw cycles of the extract. Various cell extracts are available from Active Motif (see Appendix, Section B. Related Products).

Quick Chart for Preparing Buffers and Controls

| Reagents to Prepare | Components | For 1 well | For 1 strip (8 wells) | For 6 strips (48 wells) | For 12 strips (96 wells) |
|---------------------|-------------------------|--------------------------------|---------------------------------|-------------------------|--------------------------|
| 1X Wash Buffer | Distilled water | 2.025 ml | 16.2 ml | 97.2 ml | 187.2 ml |
| | 10X Wash Buffer AMI | 225.0 μ l | 1.8 ml | 10.8 ml | 20.8 ml |
| | TOTAL REQUIRED | 2.25 ml | 18.0 ml | 108.0 ml | 208.0 ml |
| Detecting Antibody | AR antibody | 0.05 μ l | 0.45 μ l | 2.6 μ l | 5.2 μ l |
| | Diluent Buffer | 55.0 μ l | 450.0 μ l | 2.6 ml | 5.2 ml |
| | TOTAL REQUIRED | 55.05 μl | 450.5 μl | 2.6 ml | 5.20 ml |
| Secondary Antibody | HRP-conjugated antibody | 0.05 μ l | 0.45 μ l | 2.6 μ l | 5.2 μ l |
| | Diluent Buffer | 55.0 μ l | 450 μ l | 2.6 ml | 5.2 ml |
| | TOTAL REQUIRED | 55.05 μl | 450.45 μl | 2.6 ml | 5.2 ml |
| Developing Solution | TOTAL REQUIRED | 110 μl | 900 μl | 5.2 ml | 10.4 ml |
| Stop Solution | TOTAL REQUIRED | 110 μl | 900 μl | 5.2 ml | 10.4 ml |

* The above volumes include an excess of components

Assay Protocol

Read the entire protocol before use.

NR Sandwich AR Assay

Determine the appropriate number of microwell strips required for testing samples, controls and blanks in duplicate. If less than 8 wells in a strip are to be used, cover the unused wells with a portion of the plate sealer while the assay is performed. The content of these wells is stable at room temperature if kept dry. Store the unused strips in the aluminum pouch at 4°C. Use the strip holder for the assay.

Multi-channel pipettor reservoirs may be used for dispensing the 1X Wash Buffer, Antibody Binding Buffers and Developing and Stop Solution into the wells being used.

Step A: Binding of AR to the capture antibody

- Please refer to the Buffer Preparation and Recommendation section before starting the protocol.

Sample wells: Add 50 μ l of sample diluted in Diluent Buffer to each well to be used. We recommend using 5 to 50 μ g of nuclear extract diluted in Diluent Buffer per well. A protocol for preparing nuclear extracts can be found on page 10.

Control wells: Add 5 μ g of the provided LNCaP nuclear extract diluted in 50 μ l of Diluent Buffer to each well to be used (1 μ l of extract in 49 μ l of Diluent Buffer per well).

Blank wells: Add 50 μ l Diluent Buffer only per well.

2. Use the provided adhesive cover to seal the plate. Incubate for 1 hour at room temperature with mild agitation (100 rpm on a rocking platform).
3. Wash each well 3 times with 200 µl 1X Wash Buffer. For each wash, flip the plate over a sink to empty the wells, then tap the inverted plate 3 times on absorbent paper towels.

Step B: Binding of the detecting antibody

1. Add 50 µl diluted AR antibody (1:1,000 dilution in Diluent Buffer) to all wells being used.
2. Cover the plate and incubate for 1 hour at room temperature with gentle rocking.
3. Wash the wells 3 times with 200 µl 1X Wash Buffer (as described in Step 1, No. 3)

Step C: Binding of the secondary antibody

1. Add 50 µl of diluted anti-rabbit HRP-conjugated antibody (1:1,000 dilution in Diluent Buffer) to all wells being used.
2. Cover the plate and incubate for 1 hour at room temperature with gentle rocking.
3. During this incubation, place the Developing Solution at room temperature.
4. Wash the wells 4 times with 200 µl 1X Washing Buffer (as described in Step 1, No. 3).

Step D: Colorimetric detection

1. Transfer the amount of Developing Solution required for the assay into a secondary container. Add 100 µl Developing Solution to all wells being used.
2. Incubate 2-10 minutes at room temperature protected from direct light. Please read the Certificate of Analysis supplied with this kit for the optimal development time for this specific kit lot, which varies from lot to lot. Monitor the blue color development in the sample and positive control wells until it turns medium to dark blue. Do not overdevelop.
3. Add 100 µl Stop Solution. In presence of the acid, the blue color turns yellow.
4. Read absorbance on a spectrophotometer within 5 minutes at 450 nm with an optional reference wavelength of 655 nm. Blank the plate reader according to the manufacturer's instructions using the blank wells.

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Appendix

Section A. Preparation of Nuclear Extract

For your convenience, Active Motif offers a Nuclear Extract Kit (Cat. Nos. 40010 & 40410) which can be used for preparing nuclear, cytoplasmic and whole-cell extract. If you prefer to make your own buffers, please refer to the following protocol.

This procedure can be used for a confluent cell layer of 75 cm² (100 mm dish). The yield is approximately 0.5 mg of nuclear proteins for 10⁷ cells.

1. Wash cells with 10 ml of ice-cold PBS/PIB.
2. Add 10 ml of ice-cold PBS/PIB and scrape the cells off the dish with a cell lifter. Transfer the cells into a pre-chilled 15 ml tube and spin at 300 x g for 5 minutes at 4°C.
3. Resuspend the pellet in 1 ml of ice-cold HB buffer by gentle pipetting and transfer the cells into a pre-chilled 1.5 ml tube.
4. Allow the cells to swell on ice for 15 minutes.
5. Add 50 µl 10% Nonidet P-40 (0.5% final) and mix by gentle pipetting.
6. Centrifuge the homogenate for 30 seconds at 4°C in a microcentrifuge.
7. Discard the supernatant (which contains the cytoplasm and RNA) carefully without disturbing the pellet. Resuspend the nuclear pellet in 50 µl Complete Lysis Buffer and rock the tube gently on ice for 30 minutes on a shaking platform.
8. Centrifuge for 10 minutes at 14,000 x g at 4°C and save the supernatant (nuclear extract). Aliquot and store at -80°C. Avoid freeze/thaw cycles.
9. Determine the protein concentration of the extract by using a Bradford-based assay.

10X PBS

0.1 M phosphate buffer, pH 7.5
1.5 M NaCl
27 mM KCl

For 250 ml, mix:

3.55 g Na₂HPO₄ + 0.61 g KH₂PO₄
21.9 g
0.5 g

Adjust to 250 ml with distilled water. Prepare a 1X PBS solution by adding 10 ml 10X PBS to 90 ml distilled water. Sterilize the 1X PBS by filtering through a 0.2 µm filter. The 1X PBS is at pH 7.5. Store the filter-sterilized 1X PBS solution at 4°C.

PIB (Phosphatase Inhibitor Buffer)

125 mM NaF
250 mM beta-glycerophosphate
250 mM para-nitrophenyl phosphate (PNPP)
25 mM NaVO₃

For 10 ml, mix

52 mg
0.55 g
1.15 g
31 mg

Adjust to 10 ml with distilled water. Mix the chemicals by vortexing. Incubate the solution at 50°C

for 5 minutes. Mix again. Store at -20°C.

PBS/PIB

Prior to use, add 0.5 ml PIB to 10 ml 1X PBS.

HB (Hypotonic Buffer)

20 mM Hepes, pH 7.5

5 mM NaF

10 μ M Na₂MoO₄

0.1 mM EDTA

For 50 ml, mix:

0.24 g

12 mg

5 μ l of a 0.1 M solution

10 μ l of a 0.5 M solution

Adjust pH to 7.5 with 1 N NaOH. Adjust volume to 50 ml with distilled water. Sterilize by filtering through a 0.2 μ m filter. Store the filter-sterilized solution at 4°C.

Lysis Buffer

20 mM Hepes, pH 7.5

400 mM NaCl

0.1 mM EDTA

10 mM NaF

10 μ M Na₂MoO₄

1 mM NaVO₃

20% glycerol

10 mM PNPP

10 mM beta-glycerophosphate

For 50 ml, mix:

0.24 g

1.17 g

1.5 mg

21 mg

0.12 mg

6.1 mg

10 ml

0.23 g

0.11 g

Adjust pH to 7.5 with 1 N NaOH. Adjust volume to 50 ml with distilled water. Store at 4°C. Just before use, make up Complete Lysis Buffer by adding 1 μ l of 1 M DTT and 10 μ l of Protease Inhibitor Cocktail (Active Motif, Cat. Nos. 37490 & 37491) per ml of Lysis Buffer.

Section B: Troubleshooting Guide

| Problem/question | Possible cause | Recommendation |
|--|--|--|
| No signal or weak signal | Omission of key reagent | Check that all reagents have been added in all wells in the correct order |
| | Substrate or conjugate is no longer active | Test conjugate and substrate for activity by mixing HRP and Developing Solution together |
| | Enzyme inhibitor present | Sodium azide will inhibit the peroxidase reaction. Follow our recommendations to prepare buffers |
| | Plate reader settings not optimal | Verify the wavelength and filter settings |
| | Incorrect assay temperature | Bring Developing Solution and Stop Solution to room temperature before using |
| | Inadequate volume of Developing Solution | Check to make sure that correct volume is delivered by pipette |
| High background in all wells | Measurement time too long | Stop enzymatic reactions as soon as the positive wells turn medium-dark blue |
| | Concentration of antibodies is too high | Increase antibody dilutions |
| | Inadequate washing | Ensure all wells are filled with Wash Buffer and follow washing recommendations |
| Uneven color development | Incomplete washing of wells | Ensure all wells are filled with Wash Buffer and follow washing recommendations |
| | Well cross-contamination | Follow washing recommendations |
| High background in sample wells | Too much nuclear extract per well | Decrease amount of nuclear extract down to 1-2 µg/well |
| | Concentration of antibodies is too high | Perform antibody titration to determine optimal working concentration. Start using 1:2,000 for primary antibody and 1:5,000 for the secondary antibody. The sensitivity of the assay will be decreased |
| No signal or weak signal in sample wells | Not enough nuclear extract per well | Increase amount of nuclear extract. Not to exceed 50 µg/well |
| | Too many freeze/thaw cycles of extract | Aliquot extract into 5 µl aliquots and store at -80°C to avoid multiple freeze/thaws |
| | AR is poorly activated or inactivated in nuclear fractions | Perform a time course for AR activation in the studied cell line |
| | Nuclear extracts are not from correct species | Refer to cross-reactivity information on page 5 |
| | Salt concentration too high in binding reaction | Reduce amount of extract per well or dialyze extract before use |

Section C. Related Products

| Transcription Factor ELISAs | Format | Catalog No. |
|-------------------------------------|--------------------|-------------|
| TransAM™ AML-1/Runx1 | 1 x 96-well plate | 47396 |
| TransAM™ AML-3/Runx2 | 1 x 96-well plate | 44496 |
| TransAM™ AP-1 Family | 2 x 96-well plates | 44296 |
| TransAM™ AP-1 c-Fos | 1 x 96-well plate | 44096 |
| TransAM™ AP-1 c-Jun | 1 x 96-well plate | 46096 |
| TransAM™ AP-1 FosB | 1 x 96-well plate | 45096 |
| TransAM™ AP-1 JunD | 1 x 96-well plate | 43496 |
| TransAM™ ATF-2 | 1 x 96-well plate | 42396 |
| TransAM™ c-Myc | 1 x 96-well plate | 43396 |
| TransAM™ C/EBP α/β | 1 x 96-well plate | 44196 |
| TransAM™ CREB | 1 x 96-well plate | 42096 |
| TransAM™ pCREB | 1 x 96-well plate | 43096 |
| TransAM™ Elk-1 | 1 x 96-well plate | 44396 |
| TransAM™ ER | 1 x 96-well plate | 41396 |
| TransAM™ FKHR (FOXO1/4) | 1 x 96-well plate | 46396 |
| TransAM™ GATA Family | 2 x 96-well plates | 48296 |
| TransAM™ GATA-4 | 1 x 96-well plate | 46496 |
| TransAM™ GR | 1 x 96-well plate | 45496 |
| TransAM™ HIF-1 | 1 x 96-well plate | 47096 |
| TransAM™ HNF Family | 2 x 96-well plates | 46296 |
| TransAM™ HNF-1 | 1 x 96-well plate | 46196 |
| TransAM™ IRF-3 (Human) | 1 x 96-well plate | 48396 |
| TransAM™ IRF-3 (Mouse) | 1 x 96-well plate | 48496 |
| TransAM™ IRF-7 | 1 x 96-well plate | 50196 |
| TransAM™ MAPK Family | 2 x 96-well plates | 47296 |
| TransAM™ MEF2 | 1 x 96-well plate | 43196 |
| TransAM™ MyoD | 1 x 96-well plate | 47196 |
| TransAM™ NF-YA | 1 x 96-well plate | 40396 |
| TransAM™ NFATc1 | 1 x 96-well plate | 40296 |
| TransAM™ NF κ B Family | 2 x 96-well plates | 43296 |
| TransAM™ Flexi NF κ B Family | 2 x 96-well plates | 43298 |
| TransAM™ NF κ B p50 | 1 x 96-well plate | 41096 |
| TransAM™ NF κ B p50 Chemi | 1 x 96-well plate | 41097 |
| TransAM™ Flexi NF κ B p50 | 1 x 96-well plate | 41098 |
| TransAM™ NF κ B p52 | 1 x 96-well plate | 48196 |
| TransAM™ NF κ B p52 Chemi | 1 x 96-well plate | 48197 |
| TransAM™ NF κ B p65 | 1 x 96-well plate | 40096 |
| TransAM™ NF κ B p65 Chemi | 1 x 96-well plate | 40097 |
| TransAM™ Flexi NF κ B p65 | 1 x 96-well plate | 40098 |
| TransAM™ Nrf2 | 1 x 96-well plate | 50296 |
| TransAM™ Oct-4 | 1 x 96-well plate | 42496 |
| TransAM™ p53 | 1 x 96-well plate | 41196 |
| TransAM™ PPAR γ | 1 x 96-well plate | 40196 |
| TransAM™ Sp1 | 1 x 96-well plate | 41296 |
| TransAM™ Sp1/Sp3 | 1 x 96-well plate | 40496 |
| TransAM™ STAT Family | 2 x 96-well plates | 42296 |
| TransAM™ STAT3 | 1 x 96-well plate | 45196 |
| TransAM™ T-bet | 1 x 96-well plate | 51396 |

For a complete, up-to-date list of available TransAM™ Kits, please visit www.activemotif.com/transam

| Function ELISA | Format | Catalog No. |
|-------------------------------------|-------------------|-------------|
| FunctionELISA I κ B α | 1 x 96-well plate | 48005 |

| Recombinant Proteins | Format | Catalog No. |
|------------------------------|---------------|--------------------|
| Recombinant c-Fos protein | 5 µg | 31115 |
| Recombinant c-Jun protein | 5 µg | 31116 |
| Recombinant c-Myc protein | 5 µg | 31117 |
| Recombinant CREB protein | 25 µg | 31107 |
| Recombinant eIF2a protein | 25 µg | 31108 |
| Recombinant NFκB p50 protein | 5 µg | 31101 |
| Recombinant NFκB p65 protein | 5 µg | 31102 |
| Recombinant p53 protein | 5 µg | 31103 |
| Recombinant p300 protein | 4 µg | 31124 |
| Purified Sp1 protein | 2 µg | 31137 |
| Recombinant STAT3 protein | 10 µg | 31140 |

| Histone ELISAs | Format | Catalog No. |
|-----------------------------------|---------------|--------------------|
| Histone H3 monomethyl Lys4 ELISA | 1 x 96 rxns | 53101 |
| Histone H3 dimethyl Lys4 ELISA | 1 x 96 rxns | 53112 |
| Histone H3 trimethyl Lys4 ELISA | 1 x 96 rxns | 53113 |
| Histone H3 dimethyl Lys9 ELISA | 1 x 96 rxns | 53108 |
| Histone H3 trimethyl Lys9 ELISA | 1 x 96 rxns | 53109 |
| Histone H3 monomethyl Lys27 ELISA | 1 x 96 rxns | 53104 |
| Histone H3 trimethyl Lys27 ELISA | 1 x 96 rxns | 53106 |
| Histone H3 phospho Ser10 ELISA | 1 x 96 rxns | 53111 |
| Histone H3 phospho Ser28 ELISA | 1 x 96 rxns | 53100 |
| Total Histone H3 ELISA | 1 x 96 rxns | 53110 |

| Histone Purification & Chromatin Assembly | Format | Catalog No. |
|--|---------------|--------------------|
| Histone Purification Kit | 10 rxns | 40025 |
| Histone Purification Mini Kit | 10 rxns | 40026 |
| Chromatin Assembly Kit | 10 rxns | 53500 |
| HeLa Core Histones | 36 µg | 53501 |

| Co-Immunoprecipitation | Format | Catalog No. |
|-------------------------------|---------------|--------------------|
| Nuclear Complex Co-IP Kit | 50 rxns | 54001 |
| Universal Magnetic Co-IP Kit | 25 rxns | 54002 |

| DNA Methylation | Format | Catalog No. |
|-----------------------------|---------------|--------------------|
| MethylCollector™ Ultra | 30 rxns | 55005 |
| UnMethylCollector™ | 30 rxns | 55004 |
| Fully Methylated Jurkat DNA | 10 µg | 55003 |

| SUMOylation | Format | Catalog No. |
|------------------------|---------------|--------------------|
| SUMOLink™ SUMO-1 Kit | 20 rxns | 40120 |
| SUMOLink™ SUMO-2/3 Kit | 20 rxns | 40220 |

Technical Services

If you need assistance at any time, please call Active Motif Technical Service at one of the numbers listed below.

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