

RPA

DNA Repair Kits

(version B)

Catalog Nos. 51296 & 51796

Active Motif North America

1914 Palomar Oaks Way, Suite 150

Carlsbad, California 92008, USA

Toll free: 877 222 9543

Telephone: 760 431 1263

Fax: 760 431 1351

Active Motif Europe

1104 Avenue Franklin Roosevelt

B-1330 Rixensart, Belgium

UK Free Phone: 0800 169 31 47

France Free Phone: 0800 90 99 79

Germany Free Phone: 0800 181 99 10

Telephone: +32 (0)2 653 0001

Fax: +32 (0)2 653 0050

Active Motif Japan

Azuma Bldg, 7th Floor

2-21 Ageba-Cho, Shinjuku-Ku

Tokyo, 162-0824, Japan

Telephone: +81 3 5225 3638

Fax: +81 3 5261 8733

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Overview

Replication Protein A (RPA) is composed of RPA70, RPA32 and RPA14 (70, 32 and 14 kDa subunits, respectively). RPA binds directly to single stranded DNA (ssDNA) where it organizes and protects ssDNA during DNA replication, recombination and repair. The inability to replicate or repair DNA breaks leads to cell cycle arrest and apoptosis. Therefore, accurate monitoring of RPA activation in cells, tissues or animals is crucial for biomedical research and drug development. To date, such research projects are tedious and time consuming, and lack high-throughput screening methods.

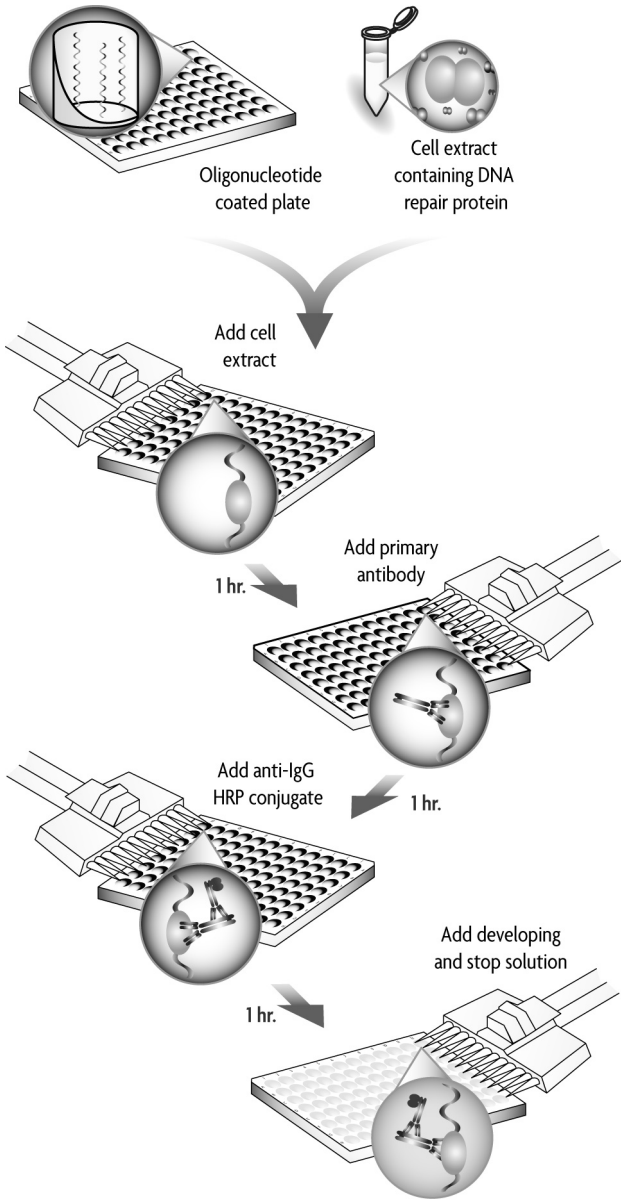
Active Motif's DNA Repair Kits* provide a fast, user-friendly format for studying DNA damage and repair protein interactions. RPA DNA Repair Kits are designed specifically for the study of RPA. They contain a 96-well plate to which a single stranded DNA oligonucleotide has been immobilized. RPA contained in nuclear extracts binds specifically to this DNA molecule and is detected through use of an antibody directed against RPA32. Addition of a secondary antibody conjugated to horseradish peroxidase (HRP) provides a sensitive colorimetric readout that is easily quantified by spectrophotometry. The 96-well plate with individual strips of 8 wells is suitable for manual use or for high-throughput screening applications. RPA DNA Repair Kits are available in two sizes:

product	format	catalog no.
RPA DNA Repair Kit	1 x 96-well plate 5 x 96 well plates	51296 51796

See Active Motif products related to RPA in Appendix, Section B.

*Patent pending

Flow Chart of Process



Introduction

RPA

The RPA protein, composed of subunits RPA70, RPA32 and RPA14, binds directly to ssDNA ends and was originally identified as a required factor for SV40 DNA replication *in vitro*.¹⁻³ More recently, it has been indicated that RPA may be involved in various DNA repair pathways. A globular domain at the C-terminus of RPA32 has been shown to interact with uracil DNA glycosylase (UDG), XPA and RAD52, each members of a different DNA repair pathway.⁴ RPA32 interacts with UDG, an important enzyme in the base excision repair (BER) pathway, and both UDG and RPA32 are known to co-localize at replication foci,^{5, 6} suggesting that RPA also plays a role in coupling BER to DNA replication. RPA32 and RPA70 bind to XPA, a key player in the nucleotide excision repair (NER) pathway, allowing interaction of XPA with sites of DNA damage.^{7, 11} Finally, RPA32 has been shown to interact with RAD52.^{8, 9} The C-terminus of RAD52 is thought to play an important role in the repair of double-stranded DNA breaks by homologous recombination.^{8, 10}

RPA is upregulated and accumulates in the nucleus after treatment with DNA damaging enediyne drugs C-1027 and neocarzinostatin.¹² The importance of RPA in DNA repair has been reported by deletion analysis as the deletion of the C-terminus of the RPA32 subunit can cause inhibition of NER activity *in vitro*.¹¹

In addition to its single-stranded DNA end binding activity, RPA has been shown to unwind double-stranded DNA.^{13, 14} RPA also binds to and stimulates the activity of helicases. RPA stimulates helicase activity of the Bloom's syndrome protein, which is involved in regulation of genomic instability and cancer predisposition.¹⁵

RPA also binds to the Werner syndrome gene product (WRN), a DNA helicase belonging to the RecQ family involved in the premature onset of age-related phenotypes.¹⁶

DNA Repair Assays

To date, two methods are widely used to measure RPA activation, either directly or indirectly:

1. RPA activation can be determined by Western blot by using antibodies specific for the RPA 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 RPA can be assayed by gel retardation, also called electrophoretic mobility shift assay (EMSA). In this method, nuclear extracts are incubated with a radioactive oligonucleotide probe. If RPA is upregulated in the cell 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.

RPA DNA Repair Kits

RPA is involved in the maintenance of genomic stability, and therefore represents an excellent pharmacological target for developing drugs to treat cancer. However, pharmaceutical research in this field has been hampered by the lack of convenient assays suitable for large numbers of samples.

To overcome this problem, Active Motif is introducing a high-throughput assay to quantify RPA activation. The DNA Repair Kit combines a fast and user-friendly ELISA format with a sensitive and specific assay for proteins involved in DNA repair. RPA DNA Repair Kits contain a 96-well plate on which has been immobilized a linear single-stranded oligonucleotide. RPA contained in nuclear extract binds specifically to this oligonucleotide. The primary antibody used in the RPA Kit recognizes an epitope on RPA32 protein that is accessible upon DNA binding. Addition of a secondary HRP-conjugated antibody provides a sensitive colorimetric readout easily quantified by spectrophotometry. Once the nuclear extracts are prepared, this assay is completed in less than 3.5 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 RPA activation and has been shown to be 20-fold faster than the gel-retardation technique. With the 3.5-hour DNA Repair Kit procedure, we could detect RPA activation using as little as 0.15 µg of nuclear extract from untreated Raji cells.

RPA DNA Repair Kits have many applications including the study of RPA regulation and protein structure/function studies of RPA in areas such as carcinogenesis and aging.

Kit Performance and Benefits

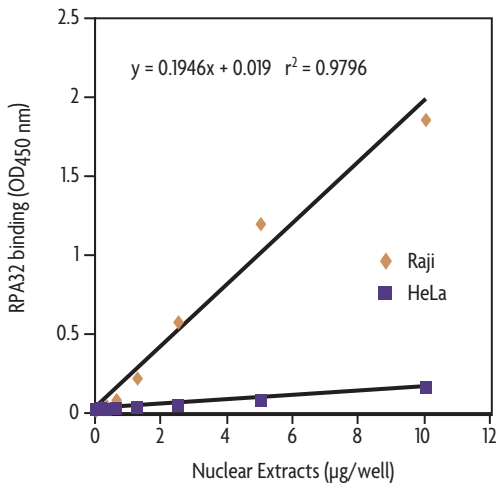
The RPA DNA Repair Kit is for research use only. Not for use in diagnostic procedures.

Detection limit: > 0.15 µg nuclear extract/well.

Range of detection: The RPA DNA Repair Kit provides quantitative results from 0.15 to 10 µg nuclear extract/well (see graph below).

Cross-reactivity: The RPA32 antibody recognizes RPA32 from human, hamster and yeast origins.

Assay time: 3.5 hours. DNA Repair Kits are 20-fold faster than EMSA.



Monitoring RPA32 binding with the RPA DNA Repair Kit: Different amounts of nuclear extracts from unstimulated Raji and HeLa cells are tested for activity using the RPA DNA Repair Kit. These curves are provided for demonstration only.

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
RPA32 antibody	11 µl / 55 µl	4°C for 1 year
Anti-mouse HRP-conjugated IgG	11 µl / 55 µl	4°C for 1 year
Competitor oligonucleotide AM23	100 µl / 500 µl (2.5 pmol/µl)	-20°C for 1 year
Raji nuclear extract	40 µl / 200 µl (2.5 µg/µl)	-80°C for 6 months
Dithiothreitol (DTT)	100 µl / 500 µl (1 M)	-20°C for 1 year
Protease Inhibitor Cocktail	100 µl / 500 µl	-20°C for 1 year
Lysis Buffer AM1	10 ml / 50 ml	4°C for 6 months
Binding Buffer AM6	10 ml / 50 ml	4°C for 6 months
10X Wash Buffer AM2	22 ml / 110 ml	4°C for 6 months
10X Antibody Binding Buffer AM2	2.2 ml / 11 ml	4°C for 6 months
Developing Solution	11 ml / 55 ml	4°C for 1 year
Stop Solution	11 ml / 55 ml	4°C for years
96-well assay plate	1 / 5	4°C for 1 year
Plate sealer	1 / 5	

Additional materials required

- Multi-channel pipettor
- Multi-channel pipettor reservoirs
- Shaking platform
- Microplate spectrophotometer capable of reading at 450 nm (655 nm as optional reference wavelength)
- Nuclear extraction reagents including Hypotonic Buffer, Detergent, Phosphatase Inhibitor Buffer and 10X PBS (see page 11)

Protocols

Buffer Preparation and Recommendations

Preparation of Complete Lysis Buffer

We provide an excess of Lysis Buffer AM1 in order to perform the assay AND to prepare customized nuclear extracts. Our Nuclear Extract Kit can also be purchased separately (Cat. Nos. 40010 & 40410). Prepare the amount of Complete Lysis Buffer required for the assay by adding 1 μ l of 1 M DTT and 10 μ l of Protease Inhibitor Cocktail per ml of Lysis Buffer AM1 (see the Quick Chart for Preparing Buffers in this section). Some of the protease inhibitors lose their activity after 24 hours once diluted. Therefore, we recommend using the Complete Lysis Buffer immediately for cell lysis. The remaining amount should be discarded if not used in the same day.

Binding Buffer AM6

This is supplied ready-to-use.

Preparation of 1X Wash Buffer

Prepare the amount of 1X Wash Buffer required for the assay as follows: For every 100 ml of 1X Wash Buffer required, dilute 10 ml 10X Wash Buffer AM2 with 90 ml distilled water (see the Quick Chart for Preparing Buffers 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 AM2 may form clumps, therefore homogenize the buffer by incubating at 50°C for 2 minutes and mixing prior to use.

Preparation of 1X Antibody Binding Buffer

Prepare the amount of 1X Antibody Binding Buffer required for the assay as follows: For every 10 ml of 1X Antibody Binding Buffer required, dilute 1 ml 10X Antibody Binding Buffer AM2 with 9 ml distilled water (see the Quick Chart for Preparing Buffers in this section)*. Mix gently to avoid foaming. Discard remaining 1X Antibody Binding Buffer after use. The BSA contained in the 10X Antibody Binding Buffer AM2 may form clumps, therefore homogenize the buffer by warming to room temperature and vortexing for 1 minute prior to use. Dilute both primary and HRP-conjugated secondary antibodies to 1:1000 with the 1X Antibody Binding Buffer. 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.

* Volumes listed refer to the preparation of buffers for diluting both the primary and secondary antibodies.

Developing Solution

The Developing Solution should be warmed to room temperature before use. The Developing 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 Developing Solution indicates that it has been contaminated and must be discarded. Prior to use, place the Developing Solution at room temperature for at least 1 hour. Transfer the amount of Developing Solution required for the assay into a secondary container before aliquoting into the wells (see the Quick Chart for Preparing Buffers in this section). After use, discard remaining Developing Solution.

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 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.

Raji nuclear extract

The Raji nuclear extract is provided as a positive control for RPA activation. Sufficient extract is supplied for 20 reactions per plate. 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).

Competitor oligonucleotide

The RPA competitor oligonucleotide is provided as a competitor for RPA binding in order to monitor the specificity of the assay. Used at 5 pmol/well, the oligonucleotide will prevent RPA binding to the probe immobilized on the plate. Prepare the required amount of competitor oligonucleotide by adding 2 µl of the oligonucleotide to 43 µl of Binding Buffer AM6 per well being used (see the Quick Chart for Preparing Buffers in this section). To allow for optimum competition, add the oligonucleotide to the well prior to addition of the nuclear extract.

Quick Chart for Preparing Buffers

Reagents to prepare	Components	For 1 well	For 1 strip (8 wells)	For 6 strips (48 wells)	For 12 strips (96 wells)
Complete Lysis Buffer	DTT	0.01 μ l	0.1 μ l	0.6 μ l	1.2 μ l
	Protease Inhibitor Cocktail	0.12 μ l	0.9 μ l	5.4 μ l	10.8 μ l
	Lysis Buffer AM1	11.12 μ l	89 μ l	534 μ l	1.068 ml
	TOTAL REQUIRED	11.25 μl	90 μl	540 μl	1.08 ml
Binding Buffer AM6	TOTAL REQUIRED	45 μl	360 μl	2.16 ml	4.32 ml
Binding Buffer AM6 with competitor oligonucleotide	Competitor oligonucleotide AM23	2.0 μ l	16.0 μ l	96.0 μ l	N/A
	Binding Buffer AM6	43.0 μ l	344.0 μ l	2.064 ml	N/A
	TOTAL REQUIRED	45.0 μl	360.0 μl	2.16 ml	N/A
1X Wash Buffer	Distilled water	2.025 ml	16.2 ml	97.2 ml	194.4 ml
	10X Wash Buffer AM2	225 μ l	1.8 ml	10.8 ml	21.6 ml
	TOTAL REQUIRED	2.25 ml	18 ml	108 ml	216 ml
1X Antibody Binding Buffer*	Distilled water	202.5 μ l	1.62 ml	9.72 ml	19.44 ml
	10X Antibody Binding Buffer AM2	22.5 μ l	180 μ l	1.08 ml	2.16 ml
	TOTAL REQUIRED	225 μl	1.8 ml	10.8 ml	21.6 ml
Developing Solution	TOTAL REQUIRED	112.5 μl	900 μl	5.4 ml	10.8 ml
Stop Solution	TOTAL REQUIRED	112.5 μl	900 μl	5.4 ml	10.8 ml

* Volumes listed refer to the preparation of buffers for diluting both the primary and secondary antibodies.

RPA DNA Repair 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 need to be used, cover the unused wells with a portion of the plate sealer while you perform the assay. The content of these wells is stable at room temperature if kept dry and, therefore, can be used later for a separate assay. Store the unused strips in the aluminum pouch at 4°C. Use the strip holder for the assay.

Prepare the Complete Lysis Buffer, 1X Wash Buffer and 1X Antibody Binding Buffer as described above in the section Buffer Preparation and Recommendations. Multi-channel pipettor reservoirs may be used for dispensing the Binding Buffer, Wash Buffer, Antibody Binding Buffer, Developing Solution and Stop Solution into the wells being used.

Step 1: Binding of RPA to the immobilized probe

1. Add 40 µl Binding Buffer AM6 to each well to be used. If you wish to perform competitive binding experiments, add 40 µl Binding Buffer AM6 that contains 5 pmol (2 µl) of the RPA competitor oligonucleotide (see the Buffer Preparation section above for a description of competitive binding).
2. **Sample wells:** Add 10 µl of sample diluted in Complete Lysis Buffer per well. We recommend using 2-10 µg of nuclear extract diluted in Complete Lysis Buffer per well.
Positive control wells: Add 5 µg of the provided Raji nuclear extract diluted in 10 µl of Complete Lysis Buffer per well (2 µl of extract in 8 µl of Complete Lysis Buffer per well).
Blank wells: Add 10 µl Complete Lysis Buffer only per well.
3. Use the provided adhesive cover to seal the plate. Incubate for 1 hour at room temperature with mild agitation (100 rpm on a Labline orbital shaker).
4. Wash each well 3 times with 200 µl 1X Wash Buffer. For each wash, flick the plate over a sink to empty the wells, then tap the inverted plate 3 times on absorbent paper towels.

Step 2: Binding of primary antibody

1. Add 100 µl diluted RPA32 antibody (1:1000 dilution in 1X Antibody Binding Buffer) to all wells being used.
2. Cover the plate and incubate for 1 hour at room temperature with mild agitation (100 rpm on a Labline orbital shaker).
3. Wash the wells 3 times with 200 µl 1X Wash Buffer (as described in Step 1, No. 4).

Step 3: Binding of secondary antibody

1. Add 100 µl of diluted anti-mouse HRP-conjugated antibody (1:1000 dilution in 1X Antibody Binding Buffer) to all wells being used.
2. Cover the plate and incubate for 1 hour at room temperature with mild agitation (100 rpm on a Labline orbital shaker).
3. During this incubation, place the Developing Solution at room temperature.
4. Wash the wells 4 times with 200 µl 1X Wash Buffer (as described in Step 1, No. 4).

Step 4: Colorimetric reaction

1. Add 100 µl Developing Solution to all wells being used.
2. Incubate 1-5 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 a reference wavelength of 655 nm. Blank the plate reader according to the manufacturer's instructions using the blank wells.

Preparation of Nuclear Extract

For your convenience, Active Motif offers a Nuclear Extract Kit (Cat. No. 40010). This kit contains buffers that are optimized to work with the DNA Repair Kit, which serves to reduce inconsistencies in the assay that may arise from using homemade or other buffers. If you prefer to make your own buffers, please refer to the following protocol.

This procedure can be used for a 15 ml cell suspension in a T75 flask. The yield is approximately 50 µg of nuclear proteins for 10^7 cells.

1. Collect 10 ml of cell suspension in a pre-chilled 15 ml tube.
2. Scrape the cells off the flask in the remaining 5 ml of media with a cell lifter. Transfer cells into the 15 ml tube and spin at 300 x g for 5 minutes at 4°C.
3. Discard supernatant. Resuspend cell pellet in 5 ml PBS/PIB and spin at 300 x g for 5 minutes at 4°C.
4. Discard supernatant. Resuspend the pellet in 1 ml ice-cold HB buffer by gentle pipetting and transfer the cells into a pre-chilled 1.5 ml tube.
5. Allow the cells to swell on ice for 15 minutes.
6. Add 50 µl 10% Nonidet P-40 (0.5 % final) and mix by gentle pipetting.
7. Centrifuge the homogenate for 30 seconds at 4°C in a microcentrifuge. Remove the supernatant (cytoplasmic fraction) and, if you wish to save this for other uses, transfer it into a pre-chilled microcentrifuge tube. (Store the cytoplasmic fraction at -80°C.)
8. Resuspend the nuclear pellet in 40 µl Complete Lysis Buffer and rock the tube gently on ice for 30 minutes on a shaking platform.
9. 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.
10. 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 Na_2HPO_4 + 0.61 g KH_2PO_4
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 β -glycerophosphate
250 mM para-nitrophenyl phosphate (PNPP)
25 mM NaVO ₃

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 of PIB in 10 ml of 1X PBS.

HB (Hypotonic Buffer)

20 mM Hepes, pH 7.5
5 mM NaF
10 μ M Na ₂ MoO ₄
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.

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Appendix

Section A. Troubleshooting Guide

PROBLEM	POSSIBLE CAUSE	RECOMMENDATION
No signal or weak signal in all wells	Omission of key reagent	Check that all reagents have been added in the correct order
	Substrate or conjugate is no longer active	Test conjugate and substrate for activity
	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 in the plate reader
	Incorrect assay temperature	Bring substrate to room temperature
	Inadequate volume of Developing Solution	Check to make sure that correct volume is delivered by pipette
High background in all wells	Developing time too long	Stop enzymatic reaction as soon as the positive wells turn medium-dark blue
	Concentration of antibodies too high	Increase antibody dilutions
	Inadequate washing	Ensure all wells are filled with Washing Buffer and follow washing recommendations
Uneven color development	Incomplete washing of wells	Ensure all wells are filled with Washing 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 2.5 µg/well
	Concentration of antibodies too high	Perform antibody titration to determine optimal working concentration. Start using 1:2000 for primary antibody and 1:5000 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 to 10 µg/well
	RPA is poorly activated or inactivated in nuclear fractions	Perform a time course for RPA activation in the studied cell line
	Cell extracts are not from human, hamster or yeast origin	Perform study with a human, hamster or yeast model

Section B. Related Products

TransAM Kits	Unit	Catalog No.
TransAM™ AP-1 Family	2 x 96 rxns	44296
TransAM™ AP-1 c-Fos	1 x 96 rxns	44096
	5 x 96 rxns	44596
TransAM™ AP-1 FosB	1 x 96 rxns	45096
	5 x 96 rxns	45596
TransAM™ AP-1 c-Jun	1 x 96 rxns	46096
	5 x 96 rxns	46596
TransAM™ NFκB Family	2 x 96 rxns	43296
TransAM™ NFκB p50	1 x 96 rxns	41096
	5 x 96 rxns	41596
TransAM™ NFκB p50 Chemi	1 x 96 rxns	41097
	5 x 96 rxns	41597
TransAM™ NFκB p65	1 x 96 rxns	40096
	5 x 96 rxns	40596
TransAM™ NFκB p65 Chemi	1 x 96 rxns	40097
	5 x 96 rxns	40597
TransAM™ Sp1	1 x 96 rxns	41296
	5 x 96 rxns	41796
TransAM™ Sp1/Sp3	1 x 96 rxns	40496
	5 x 96 rxns	40996
TransAM™ STAT Family	2 x 96 rxns	42296
TransAM™ STAT3	1 x 96 rxns	45196
	5 x 96 rxns	45696

DNA Repair Kits	Unit	Catalog No.
GTBP DNA Repair Kit	1 x 96 rxns	51096
	5 x 96 rxns	51596
Ku70/86 DNA Repair Kit	1 x 96 rxns	51196
	5 x 96 rxns	51696

Cell-based ELISAs	Unit	Catalog No.
FACE™ AKT	1 x 96 rxns	48120
	5 x 96 rxns	48620
FACE™ AKT Chemi	1 x 96 rxns	48220
	5 x 96 rxns	48720
FACE™ EGFR	1 x 96 rxns	48150
	5 x 96 rxns	48650
FACE™ EGFR Chemi	1 x 96 rxns	48250
	5 x 96 rxns	48750
FACE™ ERK1/2	1 x 96 rxns	48140
	5 x 96 rxns	48640
FACE™ ERK1/2 Chemi	1 x 96 rxns	48240
	5 x 96 rxns	48740
FACE™ JNK	1 x 96 rxns	48110
	5 x 96 rxns	48610
FACE™ JNK Chemi	1 x 96 rxns	48210
	5 x 96 rxns	48710
FACE™ p38	1 x 96 rxns	48100
	5 x 96 rxns	48600
FACE™ p38 Chemi	1 x 96 rxns	48200
	5 x 96 rxns	48700

Sandwich ELISAs	Unit	Catalog No.
FunctionELISA™ Cytochrome c	1 x 96 rxns	48006
	5 x 96 rxns	48506
FunctionELISA™ IκBα	1 x 96 rxns	48005
	5 x 96 rxns	48505
FunctionELISA™ TRAIL	1 x 96 rxns	48010
	5 x 96 rxns	48510

Supershift and Gelsift Kits	Unit	Catalog No.
Gelsift™ Sp1/Sp3	20 rxns	37322
Nushift™ p53	17 rxns	37029
Nushift™ Sp1	17 rxns	37037
Nushift™ Sp3	17 rxns	37038

Extraction and Normalization Kits	Unit	Catalog No.
Nuclear Extract Kit	100 rxns	40010
	400 rxns	40410
Nuclear Extract & Lamin Normalization Kit	1 x 96 rxns	48008
	5 x 96 rxns	48508
Mitochondrial Fractionation Kit	100 rxns	40015
GAPDH Whole-Cell Normalization Kit	1 x 96 rxns	48007
	5 x 96 rxns	48507

Extracts	Unit	Catalog No.
HeLa nuclear extract	200 µg	36010
HeLa nuclear extract (2 hr serum response)	200 µg	36104
HeLa nuclear extract (4 hr serum response)	200 µg	36086
HeLa nuclear extract (TNF-α stimulated)	200 µg	40210
HeLa nuclear extract (TPA)	200 µg	36009
HeLa S3 nuclear extract	200 µg	36038
HeLa whole-cell extract	200 µg	40050
HeLa whole-cell extract (IL-1α stimulated)	200 µg	40100
HeLa whole-cell extract (TNF-α stimulated)	200 µg	40200
Jurkat nuclear extract	200 µg	36014
Raji nuclear extract	200 µg	36023

Antibodies	Application	Unit	Catalog No.
p53 rabbit pAb	WB	100 µl	39041
p53 rabbit pAb	SS	17 rxns	39334
Sp1 rabbit pAb	WB	100 µl	39058
Sp1 rabbit pAb (crude)	WB	100 µl	39057
Sp1 rabbit pAb	SS	100 µl	39346
Sp1 rabbit pAb (crude)	SS	17 rxns	39340
Sp3 rabbit pAb	SS	17 rxns	39341

Technical Services

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

Active Motif North America

1914 Palomar Oaks Way, Suite 150

Carlsbad, CA 92008

USA

Toll Free: 877 222 9543

Telephone: 760 431 1263

Fax: 760 431 1351

E-mail: tech_service@activemotif.com

Active Motif Europe

104 Avenue Franklin Roosevelt

B-1330 Rixensart, Belgium

UK Free Phone: 0800 169 31 47

France Free Phone: 0800 90 99 79

Germany Free Phone: 0800 181 99 10

Telephone: +32 (0)2 653 0001

Fax: +32 (0)2 653 0050

E-mail: eurotech@activemotif.com

Active Motif Japan

Azuma Bldg, 7th Floor

2-21 Ageba-Cho, Shinjuku-Ku

Tokyo, 162-0824, Japan

Telephone: +81 3 5225 3638

Fax: +81 3 5261 8733

E-mail: japantech@activemotif.com

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