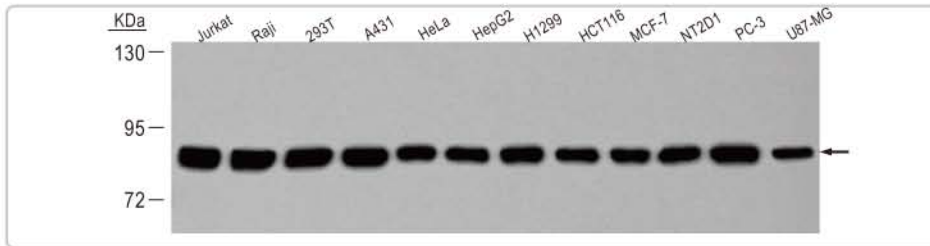
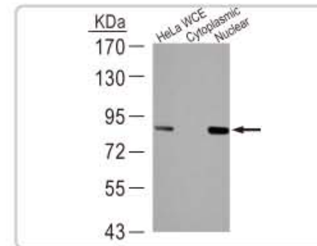
GeneTex

Your Expertise, Our Antibodies, Accelerated Discovery.

Internal Controls for Loading and Fractionation

p84 Antibody [5E10] (Nuclear Fraction or WCE)

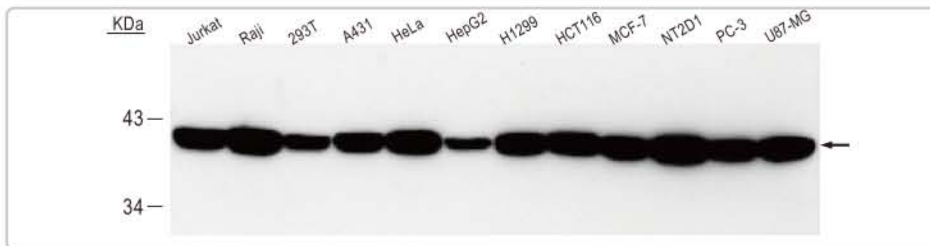


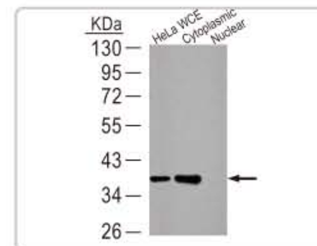


Product Information

Target:	p84 Nuclear Matrix protein
Catalog Number:	GTX70220
Clonality:	Mouse Monoclonal
Clone:	5E10
Reactivity:	Human, Mouse
Storage Buffer:	PBS, pH 7.4 with no preservatives
Concentration:	1 mg/ml
Western Blot:	1:500 – 1:3,333
ICC/IF:	1:500 – 1:2,000

GAPDH Antibody [GT239] (Cytoplasmic Fraction or WCE)





Target:	GAPDH
Catalog Number:	GTX627408
Clonality:	Mouse Monoclonal
Clone:	GT239
Reactivity:	Human, Mouse, Rat, Drosophila, Yeast
Storage Buffer:	PBS, pH 7.4 with no preservatives
Concentration:	1 mg/ml
Western Blot:	1:1,000 – 1:10,000
ICC/IF:	1:100 – 1:1,000

★ For more information or to reorder please visit **www.genetex.com/GTX300040**

Western Blot Protocol

RIPA Buffer

Tris-HCl (pH 7.6) 25 mM
NaCl 150 mM
Triton X-100 1%
Na-deoxycholate 1%
SDS 1%
Store at 4°C.
Add protease inhibitors prior to use.

Protease Inhibitors

Working concentrations:

PMSF	1 mM
Leupeptin	1 ug/ml
Aprotinin	1 ug/ml
Pepstatin	1 ug/ml

TBST

Tris-HCl (pH 7.6) 20 mM
NaCl 137 mM
Tween 20 0.1%

Blocking Buffer

5% non-fat milk in TBST

I. Lysate Preparation

Monolayer Cells

Perform the following steps on ice using pre-chilled buffers:

1. Remove culture medium from subconfluent cultures in a 100 mm cell culture plate. Rinse with PBS twice.
2. Detach cells with a rubber policeman in 1 ml cold PBS and transfer cell suspension into a 1.5 ml microcentrifuge tube.
3. Pellet cells by centrifuging at 3,000 rpm for 5 min. Remove supernatant.
4. Resuspend the pellet in 1 ml cold RIPA buffer with freshly added protease inhibitors.
5. Allow the tube to stand on ice for 30 min and vortex.
6. Centrifuge the mixture at 20,000 x g at 4°C for 30 min.
7. Transfer the supernatant to a new tube. Store lysate at -80°C.

Suspension Cells

1. Collect ~ $5x10^7$ cells by low-speed centrifugation at RT for 5 min. Carefully remove culture medium.
2. Wash the pellet with PBS at RT and collect by low-speed centrifugation. Carefully remove supernatant.
3. Add 1 ml of pre-chilled RIPA buffer with freshly added protease inhibitors. Gently resuspend cells. Incubate at 4°C for 30 min and vortex.
4. Transfer to microcentrifuge tube(s) and centrifuge at 20,000 x g at 4°C for 30 min.
5. Transfer the supernatant to a new tube. Store cell lysate at -80°C.

II. Run Lysate on SDS-PAGE Gel and Transfer to a Nitrocellulose Membrane

III. Antibody Incubation

1. Incubate the membrane in blocking buffer at RT for 30 - 60 min.
2. Incubate the membrane with primary antibody diluted in blocking buffer at 4°C overnight.
3. Wash the membrane with TBST for 5 min three times.
4. Incubate the membrane with secondary antibody diluted in blocking buffer for 1 hr.
5. Wash the membrane with TBST for 5 min three times
6. Follow the instructions of your chemiluminescence or other detection reagent.

ICC/IF Protocol

Blocking Buffer:

2.5% BSA in PBS (from the same host species as the secondary antibody)

I. Pre-treatment of Coverslips

1. Dip new cover slips into 0.1N HCl solution and keep at RT overnight.
2. Wash with three changes of water to eliminate remaining HCl.
3. Dip coverslips into 95% ethanol and keep at RT overnight.
4. Drain all of the solution and air dry the coverslips.
5. Autoclave or fire burn (immediately before using) the coverslips.

II. Cell Seeding, Fixation and Permeabilization

1. Place coverslips in a 24-well plate. Seed about 25,000 cells into the well to reach approximately 60-70% confluency the next day.
2. Remove the medium and rinse cells twice with PBS.
3. Add 1.0 ml of ice-cold methanol into the well and store the plate at -20°C for 5 - 10 min.
4. Remove the methanol, carefully add 1.0 ml PBS to the well and gently shake for 10 min.
5. Wash cells with PBS three times, each for 3 min.

III. Primary and Secondary Antibody Incubation

1. Block cells with Blocking Buffer for 30 - 60 min.
2. Dilute the primary antibody to desired dilution with blocking buffer.
3. Add 200 μL of primary antibody solution into each well and incubate at RT for 1 hr or 4°C overnight (recommended).
4. Wash cells with PBS three times, each for 3 min.
5. Add 200 μL of secondary antibody (diluted in 1% BSA/PBS) and incubate in RT for 30 - 60 min.
6. Wash cells with PBS three times, each for 3 min.
7. Note: Keep the whole plate in the dark during steps 5 and 6 if you are doing immunofluorescence.
8. If you are doing immunocytochemical staining, present the target protein by staining with appropriate kits, such as an ABC kit.

IV. Nuclear Staining and Mounting

A. For immunofluorescence, counter-stain the nuclei with DAPI for 5 min or a shorter period of time. Rinse the cells with PBS twice and then mount the coverslip onto the slide with anti-fade mounting solution.

B. For immunocytochemistry, counterstain the nucleus with hematoxylin. Rinse the cells with PBS twice and then mount the coverslIp onto the slide.

USA

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