Product Data Sheet: Phospho-ERK1/ERK2 (p44/p42 MAPK) (T202/Y204)

Cat. No:	MAB-91982
Conjugate:	Unconjugated
Size:	100 ug
Clone:	E10A
Concentration:	1mg/ml
Host:	Rb
Isotype:	IgG
Immunogen:	Synthetic phosphopeptide corresponding to residues surrounding T202/Y204 of human, mouse, and rat extracellular signal- regulated kinase-1.
Reactivity:	Hu, Ms, Rt
Applications:	Western Blot: 1:1000 IHC: 1:100 – 1:200 ICC: 1:100 IF: 1:100 IP: To be tested by the end-user
Form:	liquid
Storage:	Antibody can also be aliquotted and stored frozen at -20° C to -70° C in a manual defrost freezer for six months without detectable loss of activity. The antibody can be stored at 2° - 8° C for 1 month without detectable loss of activity. Avoid repeated freeze-thaw cycles.

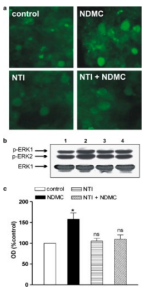

Image: Stimulation of ERK1/2 phosphorylation by NDMC in NG108-15 cells. (a) Immunofluorescence analysis of phospho-ERK1/2 immunoreactivity. Cells were serum-starved for 12 h and then treated with either vehicle (control), 10 M NDMC, 1 M NTI, and NTI+NDMC for 20 min, fixed and immunostained with anti-phospho-ERK1/2 antibody followed by FITC-conjugated secondary antibody. Results are representative of three similar experiments. (b) Western blot analysis of phospho-ERK1/2 immunoreactivity. Serum-starved cells were treated for 20 min with vehicle (lane 1), 10 M NDMC (lane 2), 1 M NTI (lane 3), and NTI+NDMC (lane 4). Thereafter, cell extracts were prepared and equal amounts of proteins (30 g) were loaded in each lane. Samples were subjected to immunoblotting with either anti-phospho-ERK1/2 antibody (top) or anti-ERK1 antibody (bottom). Results are

representative of three similar experiments. (c) Densitometric analysis of immunoreactive phospho-ERK1/2 bands. The optical density of the phospho-ERK1/2 bands for each drug treatment was normalized to the density of the corresponding ERK1 band and is reported as percent of control. Values are the mean SEM of three experiments. *p<0.05 vs control, NS p>0.05 vs control by one-way ANOVA followed by Dunnett's test.

WESTERN BLOT (WB) PROTOCOL

Western immunoblotting solutions:
Wash buffer: 1x Tris Buffered Saline (TBS); 0.1% Triton X-100 –
Blocking buffer: 1xTBS; 0.1% Triton X-100; 5% BSA (used with the primary antibody)
For western blots, incubate the membrane with antibody diluted in blocking buffer 2 hours at room temperature

IMMUNOCYTOCHEMISTRY (ICC) PROTOCOL - INSTRUCTION MANUAL

1. Coat coverslips with 1% gelatin-coating solution for 2 hours at room temperature (RT); rinse with distilled water, and let to dry overnight. Before plating the cells, wash the coated coverslips briefly with PBS. 2. Fix the cells with 4% paraformaldehyde solution (in PBS, pH 7.2), for 15 min at RT. 3. Wash 2 x 3 min with PBS. 4. Permeabilize the cells with 0.1% Triton X-100 solution (in PBS, pH 7.2) for 5 min on ice. 5. Wash 2 x 3 min with PBS. 6. Incubate the cells in blocking buffer (0.3M glycine in PBS, 2% BSA) for 30 min at RT. 7. Incubate the cells with primary antibody: anti-phospho Erk 1,2 clonal antibody at the dilution of 1:100 – in antibody dilution buffer (PBS, 1% BSA) for 1 hour at RT in humid chamber. 8. Wash 2 x 3 min with PBS. 9. Apply the secondary antibody used at 1:300 in antibody dilution buffer, and cells were incubated for 1 hour at RT in dark). 10. Wash 3 x 3 min with PBS. 11. Rinse once with distilled water. 12. Mount the slide for observation, with a drop of anti-fade mounting medium.