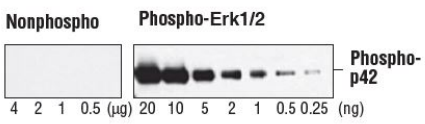
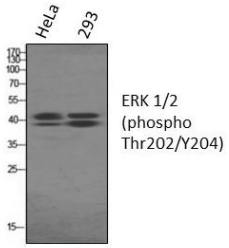
Product Data Sheet: Phospho-ERK1/ERK2 (p44/p42 MAPK) (T202/Y204)

Cat. No:	ABP-84133
Conjugate:	Unconjugated
Size:	100 ug
Clone:	Poly
Concentration:	1mg/ml
Host:	RB
Isotype:	IgG
Reactivity:	Hu, Ms, Rt
Applications:	WB 1:1000, IHC(P) 1:100, IP 1:50, IF-IC 1:100-500
Molecular Weight:	42, 44 kDa
Purification:	Polyclonal antibodies are produced by immunizing animals with a synthetic phosphopeptide cor-responding to residues surrounding Thr202/Tyr204 of human p44 MAP kinase.
Background:	Mitogen-activated protein kinases (MAPKs) are a widely conserved family of serine/threonine protein kinases involved in many cellular programs, such as cell proliferation, differentiation, motility, and death. The p44/42 MAPK (Erk1/2) signaling pathway can be activated in response to a diverse range of extracellular stimuli including mitogens, growth fac-tors, and cytokines (1-3), and research investigators consider it an important target in the diagnosis and treatment of cancer (4). Upon stimulation, a sequential three-part protein kinase cascade is initiated, consisting of a MAP kinase kinase kinase (MAPKKK or MAP3K), a MAP kinase kinase (MAPKK or MAP2K), and a MAP kinase (MAPK). Multiple p44/42 MAP3Ks have been identified, including members of the Raf family, as well as Mos and Tpl2/COT. MEK1 and MEK2 are the primary MAPKKs in this pathway (5,6). MEK1 and MEK2 activate p44 and p42 through phosphorylation of activation loop residues Thr202/Tyr204 and Thr185/Tyr187, respectively. Several downstream targets of p44/42 have been identified, including p90RSK (7) and the transcription factor Elk-1 (8,9). p44/42 are negatively regulated by a family of dual-specificity (Thr/Tyr) MAPK phosphatases, known as DUSPs or MKPs (10), along with MEK inhibitors, such as U0126 and PD98059.Phospho-p44/42 MAPK (Erk1/2) (Thr202/Tyr204) Antibody detects endogenous levels of p44 and p42 MAP Kinase (Erk1 and Erk2) when phosphorylated either individually or dually at Thr202 and Tyr204 of Erk1 (Thr185 and Tyr187 of Erk2). The antibody does not cross-react with the cor-responding phosphorylated residues of either JNK/ SAPK or p38 MAP Kinase, and does not cross-react with non-phosphorylated Erk1/2.
Form:	liquid
Buffer:	Supplied in 20 mM Tris-HCl, pH 8.0 10 mg/ml BSA ,0.05% Sodium Azide
Storage:	Store at –20°C. Do not aliquot the antibody.



Specificity and sensitivity of Phospho-

p44/42 MAPK (Erk1/2) (Thr202/Tyr204) Antibody. The antibody reacts specifically with as little as 0.25 ng of phosphorylated p42 MAP kinase and does not cross-react with up to 4 µg of nonphosphorylated p42 MAP kinase.



Anti – phospho-Erk 1,2 -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

1.Coat coverslips with 1% gelatin-coating solution for 2 hours at room temperature (RT); rinse withdistilled water, and let to dry overnight. Before plating the cells, wash the coated coverslips briefly withPBS.
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 Polyclonal antibody at the dilution of1:100 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 (in this case, the goat anti-rabbit IgG-FITC, was used at 1:300 inantibody 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.