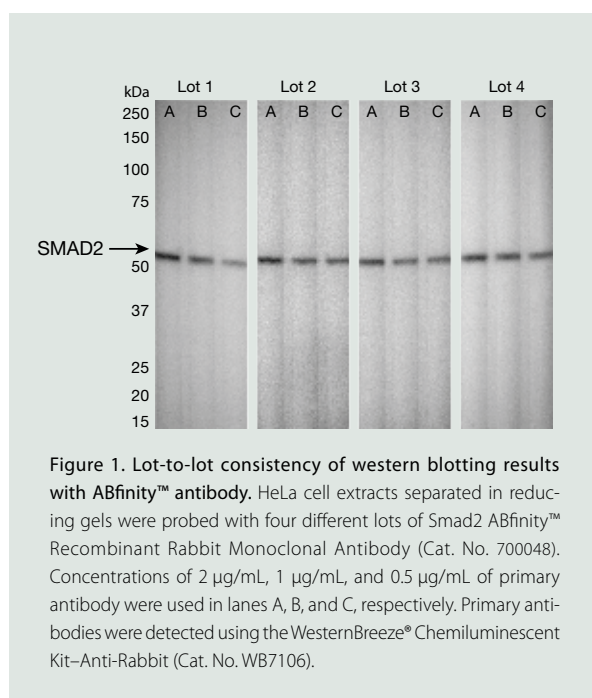


Novel recombinant monoclonal antibodies

ABFINITY™ TECHNOLOGY ENSURES PERFORMANCE.

ABfinity™ antibodies are highly specific, high-quality monoclonal antibodies that are unmatched in producing consistent results. Based on proprietary Invitrogen technology, ABfinity™ recombinant antibodies are developed by immunizing animals, screening for desired functionality, and then cloning the immunogen-specific antibody genes into high-level expression vectors. The antibodies are produced on a large scale by expressing them in mammalian cells, then highly purifying them with protein A. These recombinant antibodies can be used just like traditional IgG antibodies. The whole antibody is ~150 kDa as determined by nonreducing SDS-PAGE and, on a reducing gel, generates ~25 kDa light chain band and ~50 kDa heavy chain bands.

We offer a variety of ABfinity™ antibodies validated for various applications, including highly specific antibodies to AKT and other pathway members.

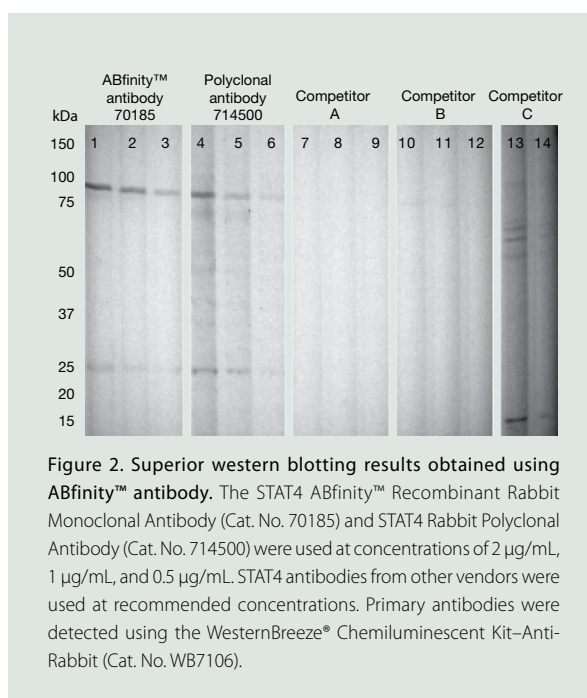


Lot-to-lot-consistency

ABfinity™ antibodies (Table 1) are manufactured by transfecting mammalian cells with heavy and light chain antibody cDNAs. This process is highly reproducible and results in unparalleled lot-to-lot consistency. This assurance of consistency saves time and money because assays do not require revalidation. Figure 1 shows the consistent western blotting results achieved using independent lots of an ABfinity™ antibody.

Reliable sensitivity and specificity

The ABfinity™ platform allows production of antibodies that are more sensitive and specific than those produced by other antibody development platforms. The high specificity of these antibodies ensures that



they only react with the target of choice, eliminating any detection of the wrong signal due to nonspecific binding. Highly sensitive antibodies can detect very low-level targets that may be difficult to detect with other antibodies. In addition, precious samples are saved by using less antibody for detection.

Figure 2 shows a direct comparison of an ABfinity™ STAT4 antibody with the best commercially available STAT4 antibodies. This comparison includes antibodies from polyclonal, traditional hybridoma monoclonal, and rabbit hybridoma monoclonal platforms.

Extensive validation and characterization

ABfinity™ antibodies are validated and characterized by multiple applications, including flow cytometry (Figure 3). This extensive validation process ensures that the antibodies can be used with confidence in target specificity and without any need for optimization.

Detecting the activated AKT kinase

PI3K/AKT is a cascading signaling pathway central to major cell functions, including cell growth, survival, apoptosis, angiogenesis, and the cell cycle.

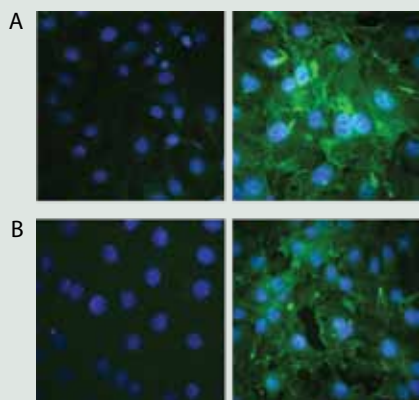


Figure 4. Immunocytochemistry analysis of mouse fibroblast cells labeled with AKT [pS473] ABfinity™ Recombinant Rabbit Monoclonal Antibody. (A) Mouse fibroblast cells were treated with (right) or without (left) 10 µg/mL insulin and labeled with AKT [pS473] ABfinity™ Recombinant Rabbit Monoclonal Antibody (Cat. No. 700392, 5 µg/mL). (B) For the insulin-treated cells in (A), the signal is knocked down after incubation with the phosphopeptide used as an immunogen (left) but not with the nonphosphopeptide (right). Alexa Fluor® 488 goat anti-rabbit IgG (Cat. No. A11008) at 1:1,000 was used as the secondary antibody. Nuclei were stained with one of the Hoechst dyes.

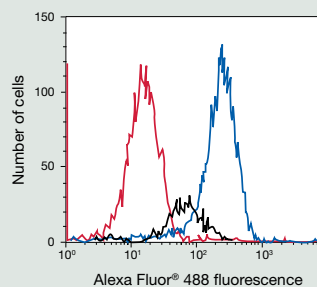


Figure 3. Flow cytometric detection of Jurkat cells labeled with JNK1/2 [pT183/pY185] ABfinity™ antibody. Jurkat cells were stimulated with 25 µg/mL anisomycin, then fixed and permeabilized using FIX & PERM® reagents (Cat. No. GAS-004). Cells were then stained with JNK1/2 antibody in the absence (blue trace) or presence (red trace) of the phosphopeptide immunogen, followed by detection using Alexa Fluor® 488 goat anti-rabbit IgG (Cat. No. A11008). The black trace represents unstimulated cells.

One of the main events in the PI3K/AKT pathway is phosphorylation of AKT at serine 473, resulting in activation of the kinase, which then acts on nuclear and cytoplasmic targets [1]. Detecting this phosphorylation event requires a very specific antibody that does not react with either unphosphorylated protein or protein phosphorylated at alternate sites. The AKT [pS473] ABfinity™ Recombinant Rabbit Monoclonal Antibody (Cat. No. 700392) recognizes the AKT protein only when it is phosphorylated at serine 473. The antibody has been validated by western blotting and can be used for rough quantification of the phosphorylated protein. Minute amounts of phosphorylated protein can be quantified using the AKT ABfinity™ antibody in a sandwich ELISA. The antibody has also been validated in immunocytochemistry and immunohistochemistry (Figures 4 and 5), allowing visualization of the cellular location of AKT in cultured cells (Figure 4) as well as in animal tissue (Figure 5). In addition, flow cytometry can be used to quantify phosphorylation at the cellular level and to separate phosphorylated and nonphosphorylated cell populations.

Other antibody tools for AKT signal transduction research

Additional ABfinity™ antibodies that have been developed for studying the effect of AKT signal transduction in glucose metabolism, the cell cycle, cell survival, adhesion, and angiogenesis are listed in Table 1. All of these antibodies are tested against multiple organisms and can be used in the applications listed in the table. We continue to offer one of the broadest portfolios of phosphorylation site-specific and total →

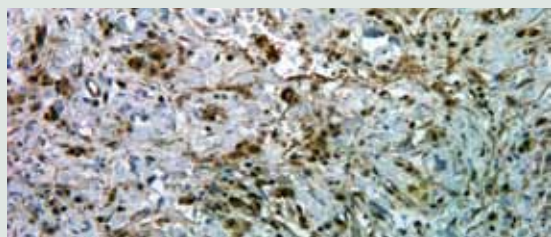


Figure 5. Immunohistochemistry analysis of human esophagus carcinoma tissue labeled with AKT [pS473] ABfinity™ Recombinant Rabbit Monoclonal Antibody. Formaldehyde-fixed, paraffin-embedded (FFPE) human esophagus carcinoma tissue was labeled with the AKT [pS473] ABfinity™ Recombinant Rabbit Monoclonal Antibody (0.5 µg/mL). The tissue was pretreated with EDTA and detected with SuperPicTure™ Polymer DAB (Cat. No. 87-8963). The image was taken at 20x magnification. Note nuclear and cytoplasmic staining in tumor cells.

antibodies. In addition, we test each lot of phosphorylation site-specific antibody by peptide competition to ensure that each lot of antibody detects only the protein phosphorylated at the correct site.

Take advantage of ABfinity™ antibody consistency

Highly specific, highly purified ABfinity™ recombinant antibodies are validated for a range of applications. For information about ABfinity™ antibodies against cell junction targets, see pages 18–19 of this issue. To view a complete list of validated Invitrogen antibodies, visit www.invitrogen.com/bp61. ■

Reference

1. Balendran A, Casamayor A, Deak M et al. (1999) *Curr Biol* 9:393–404.

Table 1. ABfinity™ antibodies.

Description	Reactivity	Applications	Quantity	Cat. No.
Antibodies to AKT and other pathway members				
AKT [pS473] ABfinity™ Recombinant Rabbit Monoclonal Antibody	Ms, Hu (Z, X, Rt, Mk, Ma, Eq, Fe, Eq, Cp, Ch, Cn, B)	E, WB, F, IHC, IF/ICC	100 µg	700392
4E-BP1 [pT37] ABfinity™ Recombinant Rabbit Monoclonal Antibody	Hu (Z, Rt, Ms, B, Eq)	E, WB, F, IHC, IF/ICC	100 µg	700238
AMPKβ1 [pS182] ABfinity™ Recombinant Rabbit Monoclonal Antibody	Hu (X, Rt, Or, Eq, Ch, Cn, B, Ms)	WB, F, IHC, IF/ICC	100 µg	700241
CASP3 [D175] ABfinity™ Recombinant Rabbit Monoclonal Antibody (clone 9H19L2)	Hu (X, Sw, Sh, Rt, Rb, P, Ms, Eq, Ha, Fe, Eq, Cp, Cn, B)	E, WB, F, IHC, IF/ICC	100 µg	700182
Cul-2 ABfinity™ Recombinant Rabbit Monoclonal Antibody	Rt, Ms, Hu (X, Or, Mk, Eq, Cp, Cn, B)	WB, F, IHC, IF/ICC	100 µg	700179
IRAK4 ABfinity™ Recombinant Rabbit Monoclonal Antibody	Hu (Sw, Sh, Rt, Qu, Eq, Cn, B)	F, IF/ICC	100 µg	700026
Mnk1 [pT197/pT202] ABfinity™ Recombinant Rabbit Monoclonal Antibody	Hu (Z, X, Sw, Rt, P, Ms, Mk, Eq, Cp, Ch, Cn, B)	WB, F, IF/ICC	100 µg	700242
PKC-θ [pT538] ABfinity™ Recombinant Rabbit Monoclonal Antibody	Hu (X, Rt, Ms, Cp, B)	WB, F, IHC, IF/ICC	100 µg	700043
Pyk2 ABfinity™ Recombinant Rabbit Monoclonal Antibody	Hu (Rt, Or, Ms, Mk, Eq, Cp, Cn, B)	F, IHC, IF/ICC	100 µg	700183
Smad1/5 [pS463/pS465] ABfinity™ Recombinant Rabbit Monoclonal Antibody	Hu (Z, X, Sw, Sh, Rt, Ms, Mk, Eq, Cp, Ch, Cn, B)	F, IHC, IF/ICC	100 µg	700047
STAT4 ABfinity™ Recombinant Rabbit Monoclonal Antibody	Rt, Ms, Hu (Sw, Eq)	E, WB, F, IHC, IF/ICC	100 µg	700185
SUMO-3 ABfinity™ Recombinant Rabbit Monoclonal Antibody	Ms, Hu, Rt (Mk, Cp)	WB, F, IHC, IF/ICC	100 µg	700186
Other ABfinity™ antibodies				
Aβ [1-42] ABfinity™ Recombinant Rabbit Monoclonal Antibody	Ms, Hu (Z, X, Sw, Rt, P, Or, Mk, Ha, Eq, Cp, Ch, Cn, B)	WB, IHC	100 µg	700254
AF-6 ABfinity™ Recombinant Rabbit Monoclonal Antibody	Ms, Hu (Mk, Cp, Cn, B, Rt)	WB, IHC	100 µg	700193
Claudin-18 ABfinity™ Recombinant Rabbit Monoclonal Antibody	Ms, Hu (Rt, Mk, Eq, Cp, Cn)	WB, IHC	100 µg	700178
c-Met ABfinity™ Recombinant Rabbit Monoclonal Antibody	Hu (P)	WB, F, IF/ICC	100 µg	700261
ERK1/2 [pT185/pY187] ABfinity™ Recombinant Rabbit Monoclonal Antibody	Hu (Z, X, Rt, Ms, Cp, Ch, B)	E, WB, IHC	100 µg	700012
IR/IGF1R [pY1162/pY1163] ABfinity™ Recombinant Rabbit Monoclonal Antibody	Hu (X, Rt, Ms, Mk, Cp, Ch, Cn, B, Eq)	E, WB	100 µg	700393
JNK1-2 [pTpY183/185] ABfinity™ Recombinant Rabbit Monoclonal Antibody (clone D12H7L17)	Hu (Z, X, Sw, Rt, Or, Ne, Ms, Mk, Gf, Eq, Cp, Ch, Cn, B)	E, WB, F, IHC, IF/ICC	100 µg	700031
PA28γ ABfinity™ Recombinant Rabbit Monoclonal Antibody	Ms, Hu, Rt (Z, X, Or, Eq, Ch, Cn)	WB, F, IHC, IF/ICC	100 µg	700180
Rab11 ABfinity™ Recombinant Rabbit Monoclonal Antibody	Hu (X, Rt, P, Ms, Eq, Ch, B)	WB, IF/ICC	100 µg	700184
Smad2 ABfinity™ Recombinant Rabbit Monoclonal Antibody (clone 31H15L4)	Hu (Z, X, Rt, Or, Ms, Gf, Ch, B)	E, WB, F, IF/ICC	100 µg	700048
T-bet ABfinity™ Recombinant Rabbit Monoclonal Antibody	Hu (Cp, Mk)	WB, F, IHC, IF/ICC	100 µg	700059
Reactivity: B = bovine; Ch = chicken; Cn = canine; Cp = chimpanzee; Eq = equine; Fe = feline; Gf = goldfish; Ha = hamster; Hu = human; Ma = mammalian; Mk = monkey (rhesus); Ms = mouse; Ne = nematode; Or = orangutan; P = primate; Qu = quail; Rb = rabbit; Rt = rat; Sh = sheep; Sw = swine; X = <i>Xenopus</i> ; Z = zebrafish. () indicates reactivity predicted but not tested. Applications: E = ELISA; F = flow cytometry; ICC = immunocytochemistry; IF = immunofluorescence; IHC = immunohistochemistry; IP = immunoprecipitation; WB = western blotting.				