

#12757 Store at 4°C

SignalFire™ Elite ECL Reagent



Orders ■ 877-616-CELL (2355)
orders@cellsignal.com
Support ■ 877-678-TECH (8324)
info@cellsignal.com
Web ■ www.cellsignal.com

rev. 06/06/18

For Research Use Only. Not For Use In Diagnostic Procedures.

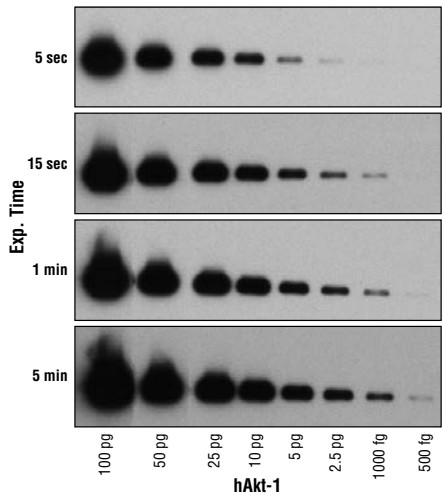
Product Includes	Product #	Quantity
12757P		
SignalFire™ Elite ECL Reagent A	94855P	10 ml
SignalFire™ Elite ECL Reagent B	17816P	10 ml
12757S		
SignalFire™ Elite ECL Reagent A	94855S	50 ml
SignalFire™ Elite ECL Reagent B	17816S	50 ml

Description: SignalFire™ Elite ECL Reagent from Cell Signaling Technology (CST) is an ultra sensitive chemiluminescent substrate capable of detecting femtomole amounts of protein by western blot analysis. SignalFire™ Elite ECL Reagent is compatible with both film and digital imaging systems. The extremely intense signal output allows detection of very low abundance proteins, conservation of reagents, and short exposure times.

SignalFire™ Elite ECL Reagent requires approximately ten-fold less Anti-rabbit IgG, HRP-linked Antibody #7074 or Anti-mouse IgG, HRP-linked Antibody #7076 than traditional ECL reagents. Limiting the amount of HRP exposed to the membrane prevents high background, oversaturation of the target protein signal, or false negative results. Other HRP-conjugated antibodies, including HRP-conjugated primary and anti-biotin-HRP antibodies, should be diluted similarly. Dilution of secondary antibody from alternative vendors may need to be optimized. Titration of lysate and primary antibody concentration is recommended to achieve optimal signal-to-noise ratio.

Background: Chemiluminescence systems have emerged as the best all-around method for western blot detection. They eliminate the hazards associated with radioactive materials and toxic chromogenic substrates. The speed and sensitivity of these methods are unequalled by traditional alternatives, and because results are generated on film, it is possible to record and store data permanently. Blots detected with chemiluminescent methods are easily stripped for subsequent reprobing with additional antibodies. HRP-conjugated secondary antibodies are utilized in conjunction with specific chemiluminescent substrates to generate the light signal. HRP conjugates have a very high turnover rate, yielding good sensitivity with short reaction times.

Solutions and Reagents: Each SignalFire™ Elite ECL Reagent A (#94855) and Reagent B (#17816) is a 2X concentrate; there is no need to further dilute in water when the two reagents are combined.



SignalFire™ Plus Elite Reagent is sensitive enough to detect femtomole levels of protein. Western blot analysis of diluted recombinant human Akt-1 protein using Akt (pan) (C67E7) Rabbit mAb #4691.

Storage: Store at 4°C. This product is stable for 12 months. DO NOT store Reagent A (#94855) and Reagent B (#17816) pre-mixed. Reagents A and B should be combined just prior to exposing membranes.

Recommended Secondary Antibody Dilutions:
Anti-rabbit IgG, HRP-linked Antibody #7074 1:10K – 1:20K
Anti-mouse IgG, HRP-linked Antibody #7076 1:10K – 1:20K

- Directions for Use:**
- (a) Wash membrane-bound HRP (antibody conjugate) at least three times for 5 minutes in TBS/T. It is very important to thoroughly wash the membrane prior to substrate incubation.
 - (b) Prepare 1X SignalFire™ Elite ECL Reagent by diluting one part 2X Reagent A (#94855) and one part 2X Reagent B (#17816) (e.g. for 10 ml, add 5 ml Reagent A and 5 ml Reagent B). Mix well.
 - (c) Incubate substrate with membrane for 1 minute, remove excess solution (membrane remains wet), wrap in plastic and expose to X-ray film.

*Avoid repeated exposure to skin (see enclosed Material Safety Data Sheet or refer to our website for further information).

© 2014 Cell Signaling Technology, Inc. SignalFire™, CST™ and Cell Signaling Technology® is a trademark of Cell Signaling Technology, Inc.

Applications Key: W—Western IP—Immunoprecipitation IHC—Immunohistochemistry ChIP—Chromatin Immunoprecipitation IF—Immunofluorescence F—Flow cytometry E-P—ELISA—Peptide
Species Cross-Reactivity Key: H—human M—mouse R—rat Hm—hamster Mk—monkey Mi—mink C—chicken Dm—D. melanogaster X—Xenopus Z—zebrafish B—bovine
Dg—dog Pg—pig Sc—S. cerevisiae Ce—C. elegans Hr—horse All—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.