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## SignalFire™ Plus ECL Reagent



**Support:** +1-978-867-2388 (U.S.)  
cellsignal.com/support

**Orders:** 877-616-2355 (U.S.)  
orders@cellsignal.com

#12630

**For Research Use Only. Not for Use in Diagnostic Procedures.**

Product Includes	Product #	Quantity
<b>12630P</b>		
SignalFire™ Plus ECL Reagent A	65293P	10 ml
SignalFire™ Plus ECL Reagent B	82201P	10 ml
<b>12630S</b>		
SignalFire™ Plus ECL Reagent A	65293S	50 ml
SignalFire™ Plus ECL Reagent B	82201S	50 ml

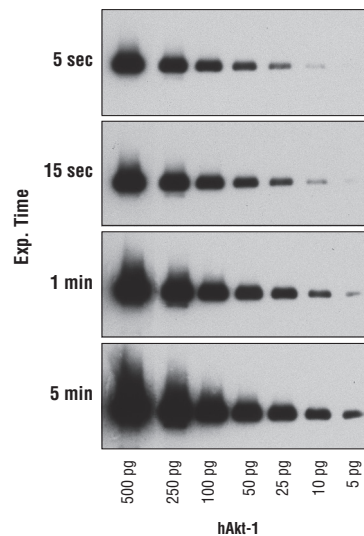
**Background:** Chemiluminescence systems have emerged as the best all-around method for western blot detection. They eliminate the hazards associated with radioactive materials and toxic chromogenic substrates. The speed and sensitivity of these methods are unequalled by traditional alternatives, and 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.

**Description:** SignalFire™ Plus ECL Reagent from Cell Signaling Technology (CST) is a highly sensitive chemiluminescent substrate capable of detecting low picogram amounts of protein by western blot analysis. SignalFire™ Plus ECL Reagent has an extended duration of signal output lasting several hours following blot exposure, allowing for multiple exposures with either film or a digital imaging system. The strong signal output allows detection of low abundance proteins, conservation of reagents, and short exposure times.

Light emission is maximal immediately after exposure of the substrate to HRP and continues for up to 24 hours.

SignalFire™ Plus ECL Reagent requires approximately five-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.

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



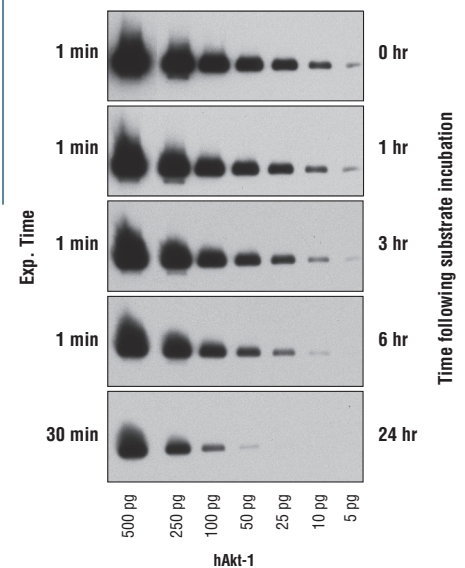
SignalFire™ Plus ECL Reagent is sensitive enough to detect low picogram amounts of protein. Western blot analysis of diluted recombinant human Akt-1 protein (hAkt-1) using Akt (pan) (C67E7) Rabbit mAb #4691.

**Storage:** Store at room temperature. DO NOT store Reagent A (#65293) and Reagent B (#82201) pre-mixed. Reagent A and B should be combined just prior to exposing membranes.

**Directions for Use:**

- 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.
- Prepare 1x SignalFire™ Plus ECL Reagent by diluting one part 2X Reagent A (#65293) and one part 2X Reagent B (#82201) (e.g. for 10 ml, add 5 ml Reagent A and 5 ml Reagent B). Mix well.
- 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).



SignalFire™ Plus ECL Reagent has extended signal duration. Western blot analysis of diluted recombinant human Akt-1 protein (hAkt-1) using Akt (pan) (C67E7) Rabbit mAb #4691.

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**Applications:** W—Western IP—Immunoprecipitation IHC—Immunohistochemistry CHIP—Chromatin Immunoprecipitation IF—Immunofluorescence F—Flow cytometry FC-FP—Flow cytometry-Fixed/Permeabilized FC-L—Flow cytometry-Live E-P—ELISA-Peptide  
**Species Cross-Reactivity:** 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.