

# SignalFire™ ECL Reagent



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

Product Includes	Product #	Quantity
<b>6883P</b>		
SignalFire™ ECL Reagent A	46935P3	25 ml
SignalFire™ ECL Reagent B	74709P3	25 ml
<b>6883S</b>		
SignalFire™ ECL Reagent A	46935S	250 ml
SignalFire™ ECL Reagent B	74709S	250 ml

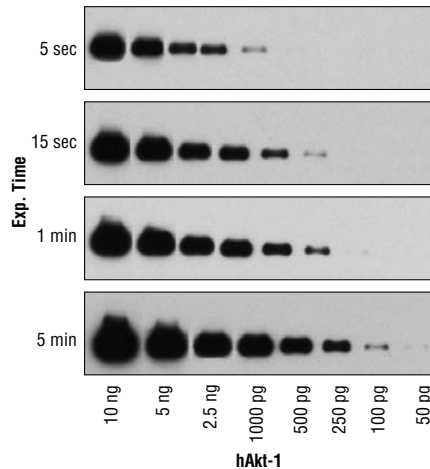
**Background:** Chemiluminescence systems have emerged as the best all-around method for western blot detection. They eliminate the hazards associated with radioactive materials and toxic chromogenic substrates. The speed and sensitivity of these methods are unequalled by traditional alternatives, and 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™ ECL Reagent from Cell Signaling Technology (CST) is an enhanced chemiluminescent substrate capable of detecting picogram amounts of protein by western blot analysis. Compared to entry-substrates, SignalFire™ ECL Reagent boasts a more robust signal and extended duration of signal output.

In the presence of hydrogen peroxide, horseradish peroxidase (HRP) converts luminol to an excited intermediate dianion. This dianion emits light on return to its ground state. Light emission is maximal immediately after exposure of the substrate to HRP and continues for 1 hour. Light can be captured on X-ray film, typically by exposure for a few seconds. Maximum sensitivity can be obtained with longer exposure.

#### Directions for Use:

- Wash membrane-bound HRP (antibody conjugate) three times for 5 minutes in TBS/T.
- Prepare 1x SignalFire™ ECL Reagent by diluting one part 2X Reagent A (#46935) and one part 2X Reagent B (#74709) (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.



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

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

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

**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 AI—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.