Chemiluminescent nucleic acid detection module, Thermo Scientific Pierce

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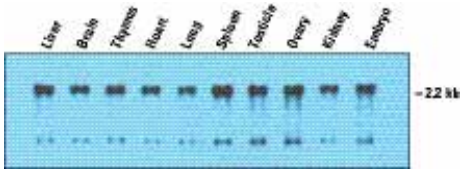
A detection system for Northerns, Southerns, gel-shifts (EMSAs) and RPAs.

The Thermo Scientific Pierce chemiluminescent nucleic acid detection module is a complete system for detection of biotin labelled nucleic acids for various blotting applications including Northern/Southern blots, ribonuclease protection assays (RPAs) and electrophoretic mobility shift assays (EMSAs).
This module provides the essential detection components used in the Thermo Scientific Pierce North2South and LightShift EMSA kits. The system includes an enhanced luminol substrate for horseradish peroxidase (HRP) with optimised blocking and wash buffers that together produce sensitivity equivalent to radioactive (^{32}P) systems.

Catalogue No	Description
PN89880	**Chemiluminescent nucleic acid detection module** Sufficient detection reagents for ten 100 x 100mm membranes. Includes: Stabilised streptavidin-horseradish peroxidase conjugate chemiluminescent, 1.5mL substrate Luminol/enhancer solution, 80mL Stable peroxide solution, 500mL Blocking buffer, 500mL 4X wash buffer, 500mL Substrate equilibration buffer, 500mL



Northern blot analysis of mouse total RNA. Total RNA samples (1µg) prepared from the indicated tissues were electrophoresed on a denaturing 1% agarose gel, transferred to Biodyne* B positive nylon, UV crosslinked and hybridised with a biotinylated c-myc/exon 3 RNA probe (4ng/mL). Exposure time to film was 1 minute.

LightShift chemiluminescent EMSA kit (gel shift), Thermo Scientific Pierce

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Identifies regulatory sequences and determines protein:DNA binding regions and affinity.

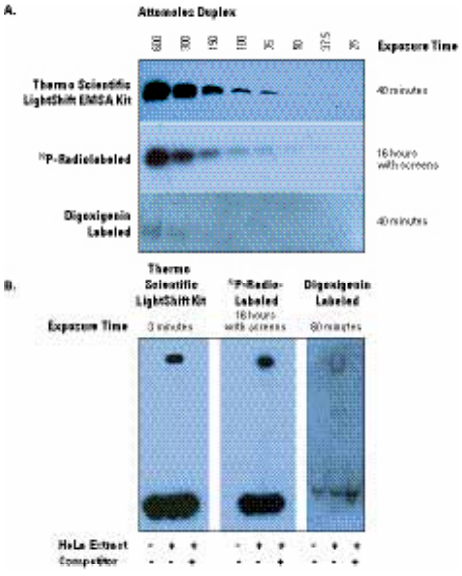
- Includes EBNA control system to help new users develop a working assay and understand the methods used to confirm binding interaction specificity
- Excellent for detecting low-abundance proteins in nuclear extracts
- Sensitivity that surpasses radioactive and digoxigenin methods
- Compatible with previously established binding conditions for popular DNA:protein interactions

The Thermo Scientific Pierce LightShift chemiluminescent EMSA kit is an extraordinarily robust and sensitive system for performing electrophoretic mobility shift assays (EMSAs) to identify and characterise protein:DNA binding interactions. The principle for LightShift™ EMSA detection is similar to a Western blot. Biotin end-labelled duplex DNA is incubated with a nuclear extract or purified factor and electrophoresed on a native gel. The DNA is then rapidly (30 minutes) transferred to a positive nylon membrane, UVcrosslinked, probed with streptavidin-HRP conjugate and incubated with the substrate. The protocol from labelling to results can be accomplished in a single day.
All you need to perform the assay are purified DNA target that has been end-labelled with biotin, the protein extract you wish to test, nylon membrane and basic electrophoresis equipment. DNA targets may be synthesised with 5′ or 3′ biotin labels or they may be labelled after synthesis using the Thermo Scientific Pierce biotin 3′ end DNA labelling kit (**PN89818**).

Catalogue No	Description
PN20148	**LightShift chemiluminescent EMSA kit** Sufficient components for 100 binding reactions and detection reagents for ~800 cm^2 of membrane. Includes: 10X binding buffer, 1mL Biotin-EBNA Control DNA, 50µL Unlabelled EBNA DNA, 50µL EBNA extract, 125µL Poly(dI•dC), 125µL 50% Glycerol, 500µL 1% NP-40, 500µL 1 M KCl, 1mL 100 mM $MgCl_2$, 500µL 200 mM EDTA, pH8.0, 500µL 5X loading buffer, 1mL Stabilised Streptavidin-Horseradish Peroxidase conjugate, 1.5mL Luminol/enhancer solution, 80mL Stable Peroxide solution, 80mL Blocking buffer, 500mL 4X wash buffer, 500mL Substrate equilibration buffer, 500mL

Accessory

Catalogue No	Description
PN89818	**Biotin 3′ end DNA labelling kit**



Comparison of the Thermo Scientific Pierce LightShift EMSA kit to a digoxigenin-based EMSA kit and a radioactive method. A) Sensitivity comparison. Serial dilutions of the labelled DNA duplexes were electrophoresed on a 6% polyacrylamide gel and detected according to the manufacturer's instructions. Comparable sensitivity (<50 attomoles) was achieved with the LightShift EMSA kit and radioactivity (2,000cpm ^{32}P/femtomole), although a significantly longer exposure was required for the ^{32}P labelled DNA. Equivalent exposures using the two chemiluminescent kits showed that the sensitivity of the LightShift EMSA kit was approximately eight-fold greater than that of the digoxigenin kit. **B) EMSA performance.** A 22bp duplex containing the binding sequence for the transcription factor Oct-1 was labelled for use in either the LightShift EMSA kit, a digoxigenin-based EMSA kit or with ^{32}P using T4 polynucleotide kinase (40,000cpm/reaction) for use in traditional radioactive EMSA. Binding reactions were equivalent in that 20fmol duplex was incubated with 6.8µg HeLa cell NE-PER Nuclear Extract (where indicated). The chemiluminescent kits were used according to the manufacturer's instructions. For the radioactive EMSA, the gel was exposed directly to X-ray film using screens.