USER GUIDE

novex® by life technologies™

Lumio™ Green Detection Kit

For specific and sensitive detection of Lumio™ fusion proteins

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life technologies™

Contents

Lumio™ Green Detection-Experienced Users Guide

Introduction

This quick reference sheet is included for experienced users of the Lumio™ Green Detection Kit. If you are a first time user, follow the detailed protocol included in this manual.

Step	Action
Prepare Samples	Prepare samples from bacterial, mammalian, or *in vitro* lysates expressing the Lumio™ fusion proteins as described on pages 8–10. You will need at least 1 pmole of the Lumio™ fusion protein for detection.
Use the Lumio™ Green Detection Kit	1. For bacterial or mammalian lysates that do not contain 1X Lumio™ Gel Sample Buffer, use 15 µL protein sample and add 5 µL 4X Lumio™ Gel Sample Buffer (see page 13 for details). For *in vitro* expressed protein samples already in 1X Lumio™ Gel Sample Buffer, use 20 µL of the sample (see page 13 for details). 2. Thaw the Lumio™ Green Detection Reagent and mix well. 3. To the protein samples from step 1, add 0.2 µL Lumio™ Green Detection Reagent using a 2 µL-pipettor. Incubate the sample at 70°C for 10 minutes. 4. Allow the samples to cool for 1–2 minutes and centrifuge briefly at high speed in a microcentrifuge. 5. Thaw the Lumio™ In-Gel Detection Enhancer and add 2 µL Lumio™ In-Gel Detection Enhancer to the samples. 6. Mix well and incubate at room temperature for 5 minutes. 7. Load 5–20 µL of the sample on an appropriate gel and perform electrophoresis. 8. After electrophoresis is complete, remove the gel from the cassette and proceed to **Visualize the Gel**.
Visualize the Gel	Place the gel on a UV transilluminator equipped with a standard camera (be sure to select the ethidium bromide or SYBR® Green filter) or use a laser-based scanner to view and capture an image of the gel. The maximum excitation wavelength for Lumio™ Green Detection Reagent is at 500 nm and maximum emission wavelength is at 535 nm (page 5). Detection with Lumio™ Green Detection Kit will result in fluorescent Lumio™ fusion protein bands (page 17).

Kit Contents and Storage

Contents and Storage

The Lumio™ Green Detection Kit is shipped on dry ice. The kit contents are described in the following table.

Sufficient reagents are included in the kit for 100 in-gel detections or 20 *in vitro* transcription-translation labeling reactions.

Reagent	Amount	Storage
Lumio™ Green Detection Reagent	20 µL	–30°C to –10°C
Lumio™ Gel Sample Buffer (4X)	5 × 200 µL	–30°C to –10°C
Lumio™ In-Gel Detection Enhancer	200 µL	–30°C to –10°C

Note

The color of the Lumio™ Green Detection Reagent may change from colorless to pink during storage. This color change does not affect the functioning of the reagent.

Product Use

For research use only. Not intended for any animal or human therapeutic or diagnostic use.

Introduction

Overview

Description

The Lumio™ Green Detection Kit uses the Lumio™ Technology to facilitate in-gel detection of Lumio™ fusion proteins. For more details on Lumio™ Technology, see page 2.

The kit is specially formulated for fast, sensitive, and specific detection, and is capable of detecting 1 pmole of a Lumio™ fusion protein (e.g. 1 pmole of a 30 kDa protein is 30 ng).

Important

Before you can use the Lumio™ Green Detection Kit to detect your protein of interest, you must express your protein using an expression construct containing your gene of interest fused to the Lumio™ sequence. A large variety of vectors are available to generate N-terminal or C-terminal Lumio™ tag fusion proteins. For more information about these vectors, visit www.lifetechnologies.com or contact Technical Support (page 22).

Features

Important features of the Lumio™ Green Detection Kit are:

- Lumio™ fusion protein sensitivity at nanogram level
- Ability to rapidly detect Lumio™ fusion proteins directly in the gel without the need for staining or western blotting
- Capable of detecting N-terminal and C-terminal Lumio™ fusion proteins
- Detection compatible with downstream applications such as Coomassie staining, silver staining, fluorescent staining, western blotting, or mass spectrometry analysis

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Lumio™ Technology

The Lumio™ Technology uses the Lumio™ Green Detection Reagent and a Lumio™ tag incorporated into the sequence of interest to facilitate fluorescent in-gel detection of Lumio™ fusion proteins.

Using the Lumio™ Technology for protein detection provides the following advantages:

- The small size of the Lumio™ tag (6 amino acids, 585 Da) is unlikely to interfere with the structure or biological activity of the protein of interest
- The Lumio™ Green Detection Reagent binds the Lumio™ tag with high specificity and high binding affinity (nanomolar or lower dissociation constant)
- The Lumio™ Green Detection Reagent is non-fluorescent until it binds the Lumio™ tag allowing specific detection of Lumio™ fusion proteins from endogenous proteins

Components of the Lumio™ System

The 2 major components of the Lumio™ System are:

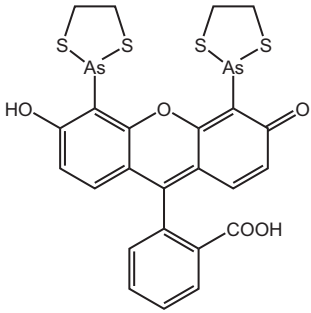
- The tetracysteine Lumio™ tag (Cys-Cys-Pro-Gly-Cys-Cys). When fused to a gene of interest (in the context of a Lumio™ vector), the Lumio™ tag specifically binds to the Lumio™ Green Detection Reagent allowing the fluorescent detection of Lumio™ fusion proteins. For more information on the tetracysteine motif, see page 3.
- A biarsenical Lumio™ Green Detection Reagent, which becomes fluorescent upon binding to recombinant proteins containing the Lumio™ tag. Lumio™ Green Detection Reagent is supplied pre-complexed to the EDT (1,2-ethanedithiol) which stabilizes and solubilizes the biarsenical reagent. For information on how the Lumio™ Green Detection Reagent binds the Lumio™ tag producing fluorescent protein bands, see page 4.

Continued on next page

Overview, Continued

Lumio™ Green Detection Reagent

The Lumio™ Green Detection Reagent is based on the FlAsH reagent (Griffin *et al.*, 1998) and is specifically formulated to allow labeling of Lumio™ fusion proteins prior to electrophoresis. The FlAsH™ (Fluorescein Arsenical Hairpin binding) reagent is a bisarsenical compound (see the following figure for the structure) used for site-specific labeling of proteins that contain a tetracysteine motif (CCXXCC), where C equals cysteine and X is any amino acid other than cysteine (Griffin *et al.*, 1998). The Lumio™ Green Detection Reagent is supplied pre-complexed to EDT (1,2-ethanedithiol) which stabilizes and solubilizes the biarsenical reagent. For information on how the Lumio™ Green Reagent binds the Lumio™ tag producing fluorescent protein bands, see page 4.



Formula: $C_{24}H_{18}As_2O_5S_4$
Molecular Weight: 664.50

Tetra-cysteine Motif

The Lumio™ Green Detection Reagent binds a tetracysteine motif consisting of Cys-Cys-Xaa-Xaa-Cys-Cys where Cys equals cysteine and Xaa equals any amino acid other than cysteine. This motif is rarely seen in naturally occurring proteins allowing specific fluorescent labeling of recombinant proteins fused to the Lumio™ tag. In the Lumio™ System, the optimized tetracysteine motif is Cys-Cys-Pro-Gly-Cys-Cys as this motif has been shown to have a higher affinity for and more rapid binding to biarsenical compounds as well as enhanced stability compared to other characterized motifs (Adams *et al.*, 2002).

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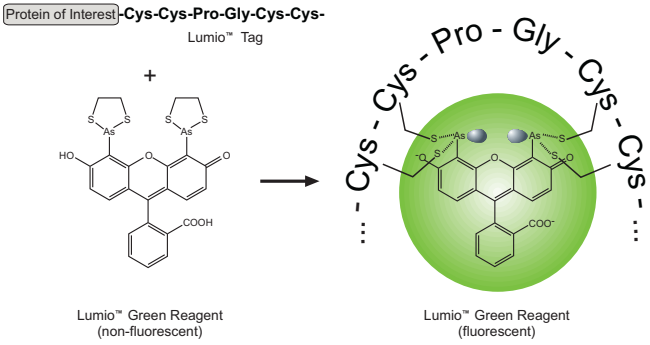
Binding of the Lumio™ Green Reagent to the Lumio™ Tag

The Lumio™ Green Detection Reagent is non-fluorescent and becomes fluorescent upon binding to the tetracysteine Lumio™ tag as follows.

When the Lumio™ Green Reagent is not bound to a protein, the small size of EDT permits free rotation of the arsenic atoms that quenches the fluorescence of fluorescein. However, when the Lumio™ Green Reagent is mixed with a Lumio™ fusion protein, the arsenic atoms of the reagent react with the tetracysteine motif of the protein and form 4 covalent bonds—the 2 arsenic groups of the Lumio™ Green Reagent each bind 2 thiols in the Lumio™ tag tetracysteine sequence (see the following figure). This binding hinders the free rotation of the arsenic atoms and the fluorescein molecule of the Lumio™ Green Reagent becomes fluorescent (increases the fluorescence by 50,000-fold). The conversion of the Lumio™ Green Reagent to a highly fluorescent state can be detected at the appropriate emission peak (see page 5). The high affinity binding of the Lumio™ Green Reagent with the Lumio™ tag allows specific detection of Lumio™ fusion proteins in the presence of endogenous proteins.



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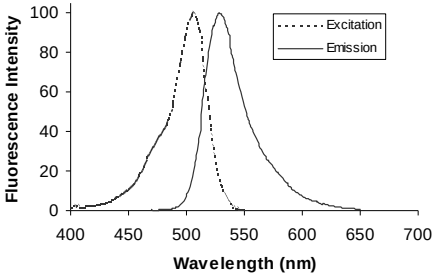
Fluorescence Spectra

The Lumio™ Green Detection Reagent has maximum excitation at 500 nm (dye can also be excited with UV light but with lower efficiency) and maximum emission at 535 nm (see the following figure). This allows you to detect the Lumio™ fusion proteins using a UV transilluminator equipped with a standard camera or a laser-based scanner.



Note

Proteins containing a C-terminal and N-terminal Lumio™ tag can be detected with the Lumio™ Green Detection Kit. However, the intensity of the signal may vary and is dependent on the individual protein.

The signal intensity is also dependent on the number of moles of protein contained in a protein band because 1 molecule of Lumio™ Green Reagent binds to only 1 Lumio™ tag on the protein.

For example, if you load 150 ng/band of 2 proteins with molecular weights of 150 kDa and 30 kDa, respectively, after detection with Lumio™ Green Detection Kit, the 30 kDa band fluoresces more intensely than the 150 kDa band. This is because there is only 1 picomole of the 150 kDa band while there are 5 picomoles of the 30 kDa band in the total mass loaded (150 ng/band).

Working with Arsenic Compounds

Introduction

Information on handling and disposing the biarsenical Lumio™ Green Detection Reagent is described below.

Exercise caution when handling the Lumio™ Green Reagent. Wear protective clothing, eyewear, and gloves suitable for use with dimethyl sulfoxide (e.g. nitrile gloves) when handling the Lumio™ Green Detection Reagent. Review the Safety Data Sheet (SDS) before handling (page 22).

Dermal Toxicity Evaluation

A dermal toxicity evaluation of the Lumio™ Green Detection Reagent was independently performed by MB Research Laboratories, Spinnerstown, PA, USA by applying a full vial of material to the mouse skin. In this study, no adverse reaction or toxicity was noted. Although arsenic compounds are toxic, this product contains <0.2% of an organic arsenic compound that shows no toxicity at a maximum dose level likely to be handled. The toxicology of this material, however, has not been fully investigated. Handle according to your chemical hygiene plan and prevent contact with this material.

Accidental Spills and Accidental Contact

Treat accidental spills of the Lumio™ Green Detection Reagent on surfaces with 10% bleach for 10 minutes and then carefully clean up. Discard arsenic-containing waste according to your institution's guidelines.

Treat accidental contact of the Lumio™ Green Detection Reagent with human skin by washing excess reagent with soap and water as soon as possible. Consult a physician following contact with Lumio™ Green Reagent. Do not treat arsenic skin exposure with EDT (1,2-ethanedithiol) as this may promote uptake of the Lumio™ Green Reagent into the body.

Properly Dispose the Lumio™ Green Reagent

All excess reagents that contain or have come in contact with arsenic compounds should be discarded according to your institution's guidelines and all applicable local, state, and federal requirements.

In general, we recommend disposing of protein samples labeled with the Lumio™ Green Detection Reagent and polyacrylamide gels containing protein samples labeled with the Lumio™ Green Detection Reagent as hazardous waste. For specific disposal requirements in your area, consult your safety officer. For SDS information, see page 22.

Experimental Overview

Introduction

The steps involved in using the Lumio™ Green Detection Kit for detecting Lumio™ fusion proteins are described below.

To visualize Lumio™ fusion protein bands after detection, you will need a UV transilluminator or a laser-based scanner. See **Visualize the Gel**, below.

Experimental Outline

The Lumio™ fusion proteins are labeled with the Lumio™ Green Detection Reagent for 10 minutes at 70°C. Then the Lumio™ In-Gel Detection Enhancer is added and the samples are incubated for 5 minutes at room temperature. The protein samples are electrophoresed and after electrophoresis, the gel is removed from the cassette. The gel is then visualized and imaged using a UV transilluminator equipped with a standard camera or a laser-based scanner (see **Visualize the Gel**, below) to visualize the fluorescent Lumio™ fusion protein bands.

The gel can be stained with Coomassie, silver, or fluorescent stains for total protein content after detection with the Lumio™ Green Detection Kit.

Visualize the Gel

For optimal visualization of the fluorescent protein bands after detection with Lumio™ Green Detection Kit, you will need:

- UV transilluminator (302 or 365 nm)

  To photograph a gel on the UV transilluminator, use a standard video camera, CCD (Charged Couple Device) camera, or a cooled CCD camera with ethidium bromide filter or SYBR® Green filter.

  Note: If you are using a 365 nm UV transilluminator, you may have to expose the gel for a longer time because the sensitivity is lower than a 302 nm UV transilluminator.

  or

- Laser-based scanner with a laser line that falls within the excitation maxima of the stain (500 nm), a 535 nm long pass filter, or a band pass filter centered near the emission maxima of 535 nm. The sensitivity of detection is higher with laser-based scanners equipped with appropriate filters than with UV transillumination.

Methods

Prepare Samples

Introduction

Brief instructions for preparing cell lysates after expression of the Lumio™ fusion proteins are described below. For details on Lumio™ fusion protein expression, see the manual supplied with the Lumio™ vector system.

Materials Needed

You will need the following items:

- Sample containing the Lumio™ fusion proteins

For bacterial lysate:

- Lysis Buffer (recipe on page 20)
- 4X Lumio™ Gel Sample Buffer (included in the kit)
- 8 M urea, optional

For *in vitro* expressed proteins:

- Acetone (cold)
- 4X Lumio™ Gel Sample Buffer (included in the kit)

For mammalian lysate:

- NP-40 Lysis Buffer (recipe on page 20)
- Protease inhibitors (optional)
- Phosphate Buffered Saline (PBS)

Note

- It is important to prepare your samples as described in this section to obtain the best results with the Lumio™ Green Detection Kit.
- Samples prepared in standard (Laemmli) SDS-PAGE sample buffer are not compatible for use with the Lumio™ Green Detection Kit.
- If you are using purified or partially purified Lumio™ fusion proteins, proceed directly to **Using Lumio™ Green Detection Kit**, page 11.

Continued on next page

Prepare Samples, Continued

Prepare Bacterial Lysates

Use the following protocol to prepare lysates from bacterial samples expressing Lumio™ fusion proteins from Champion™ pET Lumio™ vectors.

1. Thaw the cell pellets from the pilot expression (refer to the Champion™ pET Lumio™ System manual) and resuspend each pellet in 50 µL of Lysis Buffer (the recipe is on page 21).

   Note: To facilitate lysis, you may need to add lysozyme or sonicate the cells.

2. If you wish to analyze total cell lysates, proceed to **Using Lumio™ Green Detection Kit**, page 11 or store the samples at –80°C.

   If you intend to prepare lysate fractions to analyze soluble and insoluble protein, proceed to step 3.

3. Centrifuge samples at maximum speed in a microcentrifuge for 5 minutes at 4°C to pellet insoluble proteins. Transfer the supernatant (soluble protein) to a fresh tube and store on ice.

4. Wash pellets once with Lysis Buffer to remove any residual soluble proteins. Resuspend the pellets in 50 µL 8 M urea.

5. Proceed to **Using Lumio™ Green Detection Kit**, page 11.

Prepare *in vitro* Expressed Proteins

Use the following protocol to acetone-precipitate *in vitro* expressed Lumio™ fusion proteins from Expressway™ Lumio™ vectors.

1. To 5 µL of the protein reaction product (refer to the Expressway™ Lumio™ System manual), add 20 µL cold acetone. Mix well.

2. Incubate at –20°C for 20 minutes.

3. Centrifuge for 5 minutes at room temperature in a microcentrifuge at 12,000 rpm.

4. Carefully remove the supernatant, taking care not to disturb the protein pellet.

5. Resuspend the pellet in 20 µL 1X Lumio™ Gel Sample Buffer (dilute the 4X Lumio™ Gel Sample Buffer included with the kit to 1X with deionized water).

6. Proceed to **Using Lumio™ Green Detection Kit**, page 11.

Continued on next page

Prepare Mammalian Cell Lysate

Use the following protocol to prepare lysate from a 6-well plate of cells (~95% confluent) expressing Lumio™ fusion proteins from Mammalian Gateway® Lumio™ vectors.

1. Remove media from cells and wash the cells 3 times with phosphate buffered saline (PBS).
2. To each well of a 6-well plate, add 100 µL 1X NP-40 lysis buffer (the recipe is on page 20).
3. Incubate the plate for 2 minutes at room temperature.
4. Scrape cells from the bottom of the well taking care not to generate a lot of foam. Transfer the lysate into a microcentrifuge tube placed on ice.
5. Rinse the wells carefully with 50 µL 1X NP-40 lysis buffer and add it to the lysate in step 4. Keep the tube on ice.
6. For cells expressing the p64 control protein with a Lumio™ tag, we recommend sonicating the lysates. The p64 control protein localizes to the nucleoli, which are not easily lysed by the lysis buffer. Sonicate the sample 3–4 times on ice for 20 seconds each.
7. Centrifuge the lysate in a microcentrifuge at maximum speed for 2 minutes at 4°C.
8. Transfer the supernatant to a fresh microcentrifuge tube placed on ice.
9. Proceed to **Using Lumio™ Green Detection Kit**, page 11 or store the lysate at −80°C.

Using Lumio™ Green Detection Kit

Introduction

Instructions for using the Lumio™ Green Detection Kit for detecting Lumio™ fusion proteins are provided below.

For preparing lysates, see pages 8–10.

Lumio™ Gel Sample Buffer

The Lumio™ Gel Sample Buffer (4X) supplied with the kit is a proprietary sample buffer containing protein denaturing and reducing agents. The buffer is specifically formulated to provide optimal results with the Lumio™ Green Detection Reagent. **Always use the Lumio™ Gel Sample Buffer (4X) to prepare samples for electrophoresis (page 13).**

To prevent oxidation of the reducing agent in the buffer, store the Lumio™ Gel Sample Buffer (4X) at –20°C and minimize exposure to air. Use the buffer immediately upon removal from –20°C and return the buffer to –20°C immediately after use.

Lumio™ Enhancer

The Lumio™ In-Gel Detection Enhancer is a proprietary solution and is designed to reduce the non-specific binding of Lumio™ Green Detection Reagent with endogenous proteins.

BenchMark™ Fluorescent Protein Standard

The BenchMark™ Fluorescent Protein Standard (available separately, page 21) allows you to directly visualize molecular weight ranges of your Lumio™ fusion protein on a SDS-PAGE gel. For detailed information and specifications, refer to the BenchMark™ Fluorescent Protein Standard manual.

The standard consists of 7 distinct protein bands in the range of ~11–155 kDa and is supplied in a ready-to-use format. The standard proteins are easily detected using a UV transilluminator or a laser-based scanner at the same excitation and emission wavelengths as your Lumio™ fusion protein.

Signal Stability

The Lumio™ Green Detection Reagent forms a covalent bond with Lumio™ tag of the protein producing a stable Lumio™ signal. Proteins labeled with the Lumio™ Green Reagent can be stored at –20°C for up to 5 days without any loss in signal.

Continued on next page

Using Lumio™ Green Detection Kit, Continued

Materials Needed

Components required but not supplied

- Protein samples containing the Lumio™ fusion proteins
- Appropriate pre-cast gels and running buffer (page 21)
- Water bath set at 70°C
- UV transilluminator equipped with a standard camera or a laser-based scanner (see **Visualize the Gel**, page 7)

Components supplied with the kit

- Lumio™ Green Detection Reagent
- 4X Lumio™ Gel Sample Buffer
- Lumio™ In-Gel Detection Enhancer

For optimal results with the Lumio™ Green Detection Kit, follow these guidelines:

- Load at least 1 picomole of the Lumio™ fusion protein
- Use 5 µL of BenchMark™ Fluorescent Protein Standard on a mini-gel as a molecular weight marker
- **Always use the Lumio™ Gel Sample Buffer (4X) to prepare samples for electrophoresis**
- Wear protective clothing, eyewear, and gloves suitable for use with dimethyl sulfoxide (e.g. nitrile gloves) when handling the Lumio™ Green Detection Reagent
- Use the Lumio™ Gel Sample Buffer (4X) in a certified fume hood
- Use an appropriate percentage of acrylamide gel that will best resolve your protein of interest
- Visualize the gel immediately after electrophoresis to prevent diffusion of proteins as the proteins are not fixed in the gel during detection with Lumio™ Green Reagent
- Avoid touching the gel with bare hands while handling or imaging the gel
- Avoid storing the protein sample in the Lumio™ Gel Sample Buffer or Lumio™ Green Detection Reagent

Continued on next page

Procedure

After preparing the lysate, prepare samples for electrophoresis using Lumio™ Detection Reagents as described below.

1. Prepare the protein samples as follows:

Protein Sample	Sample Volume	Lumio™ Gel Sample Buffer (4X) Volume
Bacterial samples (page 9)	15 µL	5 µL
Mammalian lysate (step 8, page 10)	15 µL	5 µL
Partially purified	15 µL	5 µL
Purified sample	15 µL	5 µL
In vitro expressed (step 5, page 9)	20 µL	Not needed*

*There is no need to add Lumio™ Gel Sample Buffer (4X) since the sample is already prepared in this buffer.

2. Thaw the Lumio™ Green Detection Reagent and mix well.
3. To the protein samples from step 1, add 0.2 µL Lumio™ Green Detection Reagent using a 2 µL-pipettor (P2 pipettor) in a fume hood. Mix well. Return the Lumio™ Green Detection Reagent to –20°C immediately after use.
4. Incubate at 70°C for 10 minutes.
5. Allow the sample to cool for 1–2 minutes and centrifuge briefly at maximum speed in a microcentrifuge.
6. Thaw the Lumio™ In-Gel Detection Enhancer and mix well.
7. Add 2 µL Lumio™ In-Gel Detection Enhancer to the sample.
8. Mix well and incubate the samples at room temperature for 5 minutes. Immediately return the Lumio™ In-Gel Detection Enhancer to –20°C. You may store Lumio™ labeled proteins at –20°C for up to 5 days (page 11).
9. Load 5–20 µL of the sample on an appropriate gel and perform electrophoresis. **Note:** For NuPAGE® Novex Gels, do not add NuPAGE® Antioxidant in the running buffer for electrophoresis.
10. After electrophoresis is complete, remove gel from the cassette and proceed immediately to **Visualize and Image the Gel**, page 14. **Note:** The sensitivity of detection is much higher when the gel is visualized and imaged after removal from the cassette.

Continued on next page

Visualize and Image the Gel

Be sure to adjust the settings on the camera **prior to turning on the UV light** on the UV transilluminator. The fluorescent dye of the Lumio™ Green Reagent is sensitive to photobleaching. Avoid exposing the gel to UV light for a long time.

1. Place the gel on a UV transilluminator equipped with a standard camera and make sure the ethidium bromide or SYBR® Green filter is selected on the camera.

   You may also use a laser-based scanner with a laser line that falls within the excitation maxima of the stain (500 nm), and a 535 nm long pass filter or a band pass filter centered near the emission maxima of 535 nm (pages 5 and 7 for more details).

2. Image the gel with a suitable camera with the appropriate filters using 4–10 second exposure. You may need to adjust the brightness and contrast to reduce any faint non-specific bands.

   You should see fluorescent bands of Lumio™ fusion proteins and the gel should have minimal background as shown on page 17.

   Note: The Lumio™ fusion protein bands appear white or black depending on the type of imaging system used for imaging the gels.

Note

- The fluorescent signal is stable for 10–15 minutes, if the gel is not exposed to UV light.
- The fluorescence emission of the Lumio™ Green Detection Reagent is in the green light region. If you have a suitable imaging system with a colored camera and appropriate filters, you maybe able to visualize and image the emitted green fluorescence.
- Longer exposure times may produce a fluorescent dye front.

Important

The detection with Lumio™ Green Detection Kit is not permanent and is lost by subsequent staining of the gel with other protein stains. It is extremely important to record a permanent image of the gel prior to staining the gel with protein stains and gel drying.

Continued on next page

Stain the Gel for Total Protein

After detecting the Lumio™ fusion proteins, the gel can be stained with SimplyBlue™ SafeStain (Coomassie G-250 stain), Coomassie R-250 stain, or a fluorescent protein stain such as SYPRO® Ruby Protein Gel Stain (see page 21 for ordering information) to view the total protein content of the sample.

1. Record a permanent image of the gel after detecting the Lumio™ fusion proteins.
2. Follow the appropriate staining protocol described below.

SimplyBlue™ SafeStain Procedure

1. Rinse the mini-gel 3 times for 5 minutes with 100 mL deionized water.
2. Stain the gel for 1 hour with gentle shaking at room temperature with enough SimplyBlue™ SafeStain (page 21 for ordering information) to cover the gel.
3. Wash the gel with 100 mL deionized water for 1–3 hours.

Coomassie R-250 Staining Procedure

You may use any Coomassie R-250 staining procedure of choice. Perform fixing, staining, and destaining steps using a procedure of choice.

Fluorescent Protein Staining Procedure

Follow the instructions in the product manual to stain and view the protein bands on the gel using SYPRO® Ruby Protein Gel Stain (see page 21 for ordering information).

Western Blot

To perform western blotting and immunodetection after using the Lumio™ Green Detection of Lumio™ fusion proteins:

1. Record a permanent image of the gel after detecting the Lumio™ fusion proteins.
2. Perform western blotting and immunodetection using a method of choice.

To visualize Lumio™ fusion proteins transferred onto a nitrocellulose or PVDF membrane, see **Detect Lumio™ Fusion Proteins on a Blot**.

Continued on next page

Using Lumio™ Green Detection Kit, Continued

Detect Lumio™ Fusion Proteins on a Blot

Visualize the Lumio™ fusion protein transferred onto a nitrocellulose or PVDF membrane using any method described below.

- Use a hand held UV lamp to view proteins on the membrane.
- Place the membrane on a UV transilluminator equipped with a standard camera. Visualize and image the membrane by exposing the membrane to UV light from the bottom or by exposing the membrane to UV light from the top (you may place the UV transilluminator on its side to illuminate the blot or use a top illuminating system).
- Use a laser-based scanner with appropriate filters to visualize and image the membrane by epi-illumination (reflective).

Prepare Protein Bands for MS Analysis

Proteins detected with the Lumio™ Green Detection Kit are compatible with mass spectrometry (MS) analysis.

After detection with Lumio™ Green Detection Kit, excise the protein band/spot and prepare your samples for MS analysis using a method of choice or as directed by your core facility.

The Lumio™ Green Detection protocol produces the following protein modifications. Be sure to account for these during MS analysis.

- Cysteines in the protein are modified and will result in the addition of 97.07 Da to each cysteine.
- The total molecular weight of the Lumio™ tag with the Lumio™ Green Reagent is 1060 Da (molecular weight of the Lumio™ Green Reagent without EDT is 475 Da and molecular weight of the Lumio™ tag is 585 Da).

Expected Results

Results

An example of Lumio™ Green Detection of Lumio™ fusion proteins using the procedure described on page 14 is shown below.

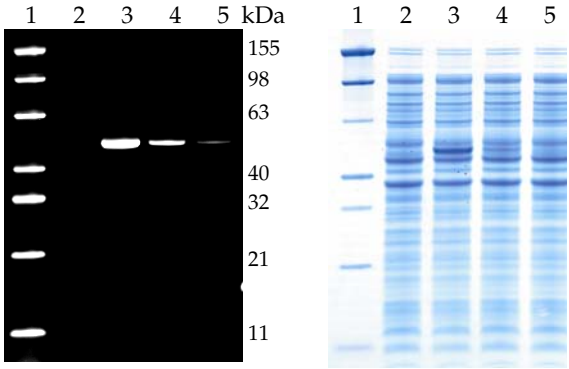
Protein samples were labeled using the Lumio™ Green Detection Kit and electrophoresed on a NuPAGE® Novex 4–12% Bis-Tris Gel. The Lumio™ fusion protein signal was detected and imaged on a UV transilluminator (302 nm) equipped with a standard camera (A), subsequently the gel was Coomassie stained for total protein detection with SimplyBlue™ SafeStain (B).

Lane 1: 5 µL BenchMark™ Fluorescent Protein Standard

Lane 2: BL21 Star™ *E. coli* extract-negative control

Lanes 3–5: 1200 ng, 240 ng, 48 ng, respectively, of pure 48 kDa Lumio™ fusion protein mixed with BL21 Star™ *E. coli* lysate

Lumio™ Green Detection (A) **SimplyBlue™ SafeStain (B)**



Troubleshooting

Introduction

Solutions for possible problems you might encounter while using the Lumio™ Green Detection Kit are listed in the following table.

Observation	Cause	Solution
No bands or weak signal	Check labeling protocol	• Be sure to follow the labeling protocol as described on page 13 to obtain the best results. • Make sure you have added the Lumio™ Green Detection Reagent to the samples prior to electrophoresis. • Limit exposure of the Lumio™ Gel Sample Buffer (4X) to air. Always return the Lumio™ Green Reagent and Lumio™ Enhancer to –20°C immediately after use to preserve the activity of buffers.
	Low protein load or low expression level	• Check total protein loaded on the gel by staining the gel with a total protein stain (page 15). Load at least 1 pmole of the Lumio™ fusion protein. • Make sure the Lumio™ tag is in frame and the protein is expressed properly. A positive control is supplied with the Lumio™ vectors to verify the expression protocol.
	The gel is exposed to UV light for a long time	Avoid exposing the gel to UV light for a long time. The fluorescent dye of the Lumio™ Green Reagent is sensitive to photobleaching.
	The gel is not visualized immediately or imaged properly	Be sure to visualize the gel after removing the gel from the cassette and view the gel immediately after electrophoresis. Use a UV transilluminator or a laser-based scanner using appropriate filters (page 7). **Tip:** If you have run BenchMark™ Fluorescent Protein Standard on the same gel and can view the standard bands on the gel, then you are imaging the gel properly.

Continued on next page

Troubleshooting, Continued

Observation	Cause	Solution
High or uneven background	Improper handling of gels or dirty imaging platform	• Avoid touching the gel with bare hands while handling or imaging the gel. • Always clean the imaging platform with a paper towel prior to imaging the gel to minimize any background fluorescence.
	Protein overloaded	Decrease the protein concentration or lower the sample volume.
	Non-specific bands	• Use the Lumio™ In-Gel Detection Enhancer to minimize non-specific binding. • Certain proteins from *E. coli* lysates (SlyD, 21 kDa) and serum proteins (BSA, 66 kDa) from the mammalian cell culture medium may cross-react with the Lumio™ Green Reagent producing non-specific bands. Removing the cell culture medium and washing the mammalian cells 3–4 times with PBS after harvesting the cells minimizes the non-specific binding from BSA.

Appendix

Recipes

NP-40 Lysis Buffer (5X)

Mix the following items to prepare 200 mL of 5X NP-40 Lysis Buffer:

5 M NaCl	30 mL
NP-40 (Igepal CA-630, Sigma #I-3021)	10 mL
1 M Tris, pH 8.0	50 mL
Water	110 mL
Total Volume	200 mL

Store the buffer at room temperature.

Immediately before use, dilute 5X NP-40 buffer to 1X with deionized water and use this buffer to prepare lysates from mammalian cells (page 10). Add protease inhibitors (optional, but recommended).

Lysis Buffer

50 mM potassium phosphate, pH 7.8
400 mM NaCl
100 mM KCl
10% glycerol
0.5% Triton® X-100
10 mM imidazole

1. Prepare 1 M stock solutions of KH_2PO_4 and K_2HPO_4.
2. For 100 mL, dissolve the following reagents in 90 mL of deionized water:

   0.3 mL 1 M KH_2PO_4
   4.7 mL 1 M K_2HPO_4
   2.3 g NaCl
   0.75 g KCl
   10 mL glycerol
   0.5 mL Triton® X-100
   68 mg imidazole
3. Mix thoroughly and adjust the pH to 7.8 with HCl. Bring the volume to 100 mL.
4. Store at 4°C.

Accessory Products

Additional Products

Additional products are available separately. Ordering information is listed in the following table. For more details, visit www.lifetechnologies.com or contact Technical Support (page 22).

Product	Quantity	Catalog no.
BenchMark™ Fluorescent Protein Standard	125 µL	LC5928
NuPAGE® Novex 4-12% Bis-Tris Gels	10 gels	NP0321BOX
Novex 10% Tris-Glycine Gels	10 gels	EC6075BOX
XCell *SureLock*® Mini-Cell	1 unit	EI0001
SimplyBlue™ SafeStain	1 L	LC6060
SilverQuest™ Silver Staining Kit	1 kit	LC6070
SYPRO® Ruby Protein Gel Stain	1 L	S-12000

Lumio™ Vectors

A large variety of vectors are available to generate N-terminal or C-terminal Lumio™ fusion proteins. For more information about these vectors, visit www.lifetechnologies.com or contact Technical Support (page 22).

Technical Support

Obtaining support

For the latest services and support information for all locations, go to **www.lifetechnologies.com/support**.

At the website, you can:

- Access worldwide telephone and fax numbers to contact Technical Support and Sales facilities
- Search through frequently asked questions (FAQs)
- Submit a question directly to Technical Support (**techsupport@lifetech.com**)
- Search for user documents, Safety Data Sheets (SDSs), vector maps and sequences, application notes, formulations, handbooks, certificates of analysis, citations, and other product support documents
- Obtain information about customer training
- Download software updates and patches

Safety Data Sheets (SDS)

Safety Data Sheets (SDSs) are available at **www.lifetechnologies.com/support**.

Certificate of Analysis

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Continued on next page

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