USER GUIDE

invitrogen™
by life technologies™

LiveBLAzer™ FRET — B/G Loading Kit

with CCF2-AM and CCF4-AM

Catalog Number K1023, K1025, K1028, K1029, K1030, K1032, K1089, K1095, and K1096

Publication Number MAN0002771

Revision A.0

For Research Use Only. Not for use in diagnostic procedures.

life technologies™

For Research Use Only. Not for use in diagnostic procedures.

The information in this guide is subject to change without notice.

DISCLAIMER

LIFE TECHNOLOGIES CORPORATION AND/OR ITS AFFILIATE(S) DISCLAIM ALL WARRANTIES WITH RESPECT TO THIS DOCUMENT, EXPRESSED OR IMPLIED, INCLUDING BUT NOT LIMITED TO THOSE OF MERCHANTABILITY, FITNESS FOR A PARTICULAR PURPOSE, OR NON-INFRINGEMENT. TO THE EXTENT ALLOWED BY LAW, IN NO EVENT SHALL LIFE TECHNOLOGIES AND/OR ITS AFFILIATE(S) BE LIABLE, WHETHER IN CONTRACT, TORT, WARRANTY, OR UNDER ANY STATUTE OR ON ANY OTHER BASIS FOR SPECIAL, INCIDENTAL, INDIRECT, PUNITIVE, MULTIPLE OR CONSEQUENTIAL DAMAGES IN CONNECTION WITH OR ARISING FROM THIS DOCUMENT, INCLUDING BUT NOT LIMITED TO THE USE THEREOF.

Important Licensing Information

These products may be covered by one or more Limited Use Label Licenses. By use of these products, you accept the terms and conditions of all applicable Limited Use Label Licenses.

All trademarks are the property of Thermo Fisher Scientific and its subsidiaries unless otherwise specified. Pluronic is a registered trademark of BASF Corporation.

©2014 Thermo Fisher Scientific Inc. All rights reserved.

Contents

About This Guide

IMPORTANT! Before using this product, read and understand the information in the "Safety" appendix in this document.

Revision history

Revision	Date	Description
A.0	March 2014	• Removed obsolete and discontinued products • Updated to current style

Product information

Product description

Beta-lactamases comprise a family of bacterial enzymes that cleave penicillins and cephalosporins. These enzymes have been extensively studied with respect to their three-dimensional structure, mechanism and substrate specificity. One commonly studied isoform is the 29-kDa, plasmid-encoded TEM-1 β-lactamase from *E. coli* that is the product of the ampicillin resistance gene, *AmpR*. A modified version of this compact, efficient enzyme was identified as an excellent candidate for a reporter gene in mammalian cells, where it is not naturally found.

The development of two Fluorescence Resonance Energy Transfer (FRET)-based fluorescent substrates, CCF2 and CCF4, which consist of a cephalosporin core linking a 7-hydroxycoumarin to a fluorescein, permits the use of β-lactamase as a sensitive reporter of mammalian gene expression. The lipophilic, esterified form of this substrate (CCF2-AM or CCF4-AM) readily enters the cell. Cleavage by endogenous cytoplasmic esterases rapidly converts CCF2-AM or CCF4-AM into its negatively charged form, CCF2 or CCF4, which is retained in the cytosol. This substrate offers a simple and flexible assay development platform for transcriptional readouts in mammalian cells. Major advantages of this reporter gene system include its use in live cell experiments, ratiometric readout, compatibility with flow cytometry, and utility for high-throughput screening (HTS) microplate-based transcriptional assays.

The design of a fluorescent substrate for β-lactamase was based on the observation that cleavage of the β-lactam ring of a cephalosporin results in the spontaneous elimination of any leaving group attached to the 3'-position. By labeling this position and a second site in the cephalosporin molecule with two fluorophores that exhibit efficient FRET, it was possible to develop a substrate where FRET is disrupted upon cleavage (Figure 1).

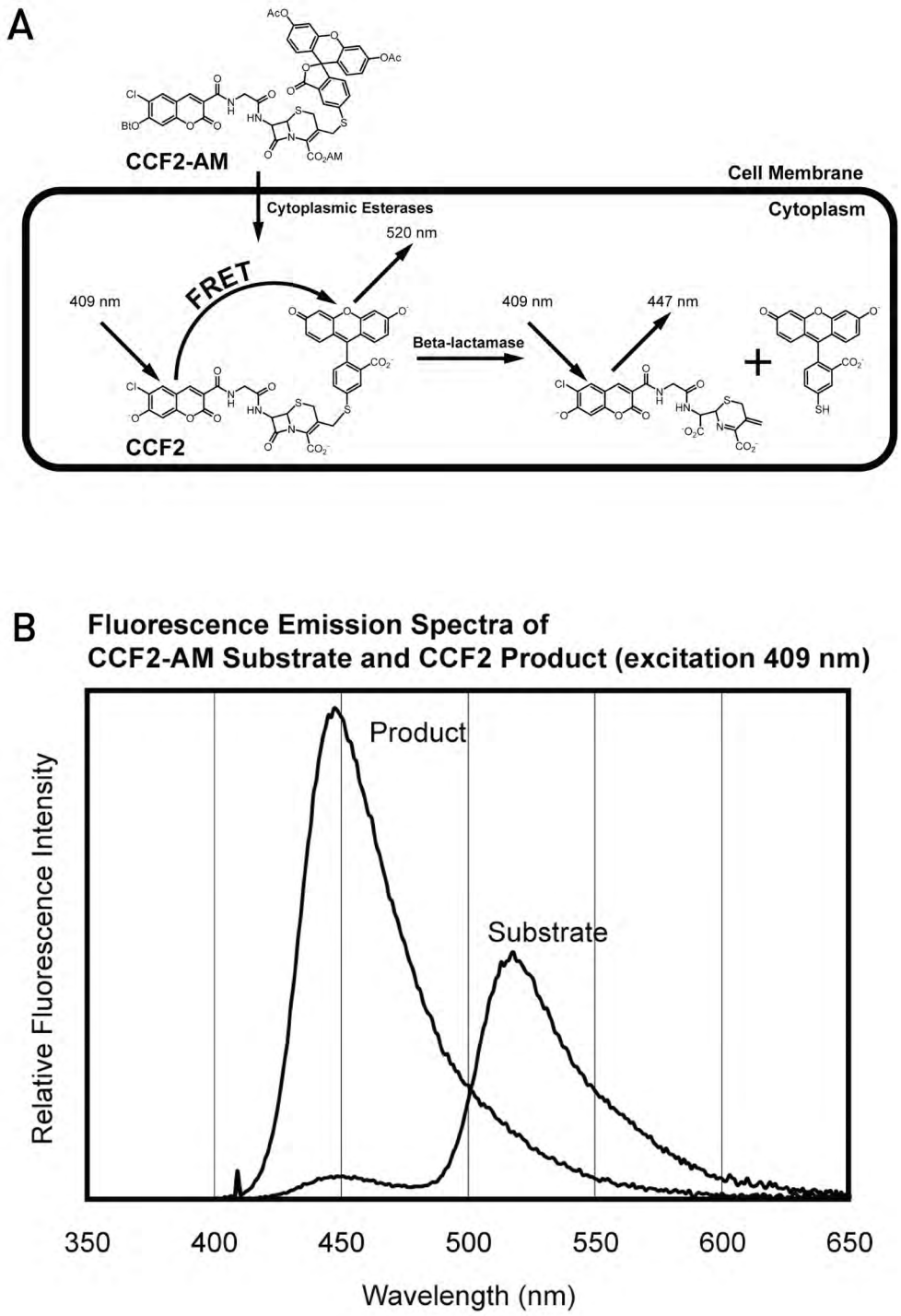


Figure 1 The lipophilic, esterified form of CCF2 or CCF4 readily enters the cell. Cleavage by endogenous cytoplasmic esterases rapidly converts this molecule into the negatively-charged substrate, which is retained in the cytosol. In the absence of β-lactamase activity, excitation of the coumarin (at 409 nm) in the intact molecule, results in FRET to the fluorescein, which emits a green fluorescence signal (at 520 nm) (**Panel A**). In the presence of β-lactamase, enzymatic cleavage of CCF2 or CCF4 spatially separates the two dyes and disrupts FRET, so that excitation of the coumarin (at 409 nm) now produces a blue fluorescence signal (450 nm) (**Panel A and B**).

LiveBLAzer™ FRET — B/G Loading Kit contents and storage

Loading kit with CCF2-AM (Cat no. K1025, 5 mg)				
Component	**Cat. no./ Part. no.**	**Description**	**Quantity**	**Storage**
CCF2-AM [1]	**K1023**	Dried down under vacuum from acetonitrile.	5 mg	–30°C to –10°C; Desiccate and protect from light.
DMSO for Solution A	K1035	—	10 mL	15°C to 30°C; Protect from direct light.
Solution B	K1036	100 mg/mL Pluronic®-F127 surfactant in DMSO and 0.1% acetic acid.	75 mL	
Solution C	K1037	24% w/w PEG 400, 18% TR-40 by volume in water.	800 mL	

Loading kit with CCF2-AM (Cat no. K1032, 200 µg)				
Component	**Part. no.**	**Description**	**Quantity**	**Storage**
CCF2-AM	K1039	Dried down under vacuum from acetonitrile.	200 µg	–30°C to –10°C; Desiccate and protect from light.
DMSO for Solution A	K1040	—	1 mL	15°C to 30°C; Protect from direct light.
Solution B	K1041	100 mg/mL Pluronic®-F127 surfactant in DMSO and 0.1% acetic acid.	5 mL	
Solution C	K1048	24% w/w PEG 400, 18% TR-40 by volume in water.	32 mL	

Loading kit with CCF4-AM (Cat no. K1030, 5 mg)				
Component	**Cat. no./ Part. no.**	**Description**	**Quantity**	**Storage**
CCF4-AM [2]	**K1028**	Dried down under vacuum from acetonitrile.	5 mg	–30°C to –10°C; Desiccate and protect from light.
DMSO for Solution A	K1035	—	10 mL	15°C to 30°C; Protect from direct light.
Solution B	K1036	100 mg/mL Pluronic®-F127 surfactant in DMSO and 0.1% acetic acid.	75 mL	
Solution C	K1037	24% w/w PEG 400, 18% TR-40 by volume in water.	800 mL	

[1] CCF2-AM is also available separately (Cat. no. K1023)
[2] CCF4-AM is also available separately (Cat. no. K1028)

Loading kit with CCF4-AM (Cat no. K1096, 1 mg)				
Component	**Part. no.**	**Description**	**Quantity**	**Storage**
CCF4-AM	K1089	Dried down under vacuum from acetonitrile.	5 × 200 µg	–30°C to –10°C; Desiccate and protect from light.
DMSO for Solution A	K1040	—	5 × 1 mL	15°C to 30°C; Protect from direct light.
Solution B	K1041	100 mg/mL Pluronic®-F127 surfactant in DMSO and 0.1% acetic acid.	5 × 5 mL	
Solution C	K1048	24% w/w PEG 400, 18% TR-40 by volume in water.	5 × 32 mL	

Loading kit with CCF4-AM (Cat no. K1095, 200 µg)				
Component	**Part. no.**	**Description**	**Quantity**	**Storage**
CCF4-AM	K1089	Dried down under vacuum from acetonitrile.	200 µg	–30°C to –10°C; Desiccate and protect from light.
DMSO for Solution A	K1040	—	1 mL	15°C to 30°C; Protect from direct light.
Solution B	K1041	100 mg/mL Pluronic®-F127 surfactant in DMSO and 0.1% acetic acid.	5 mL	
Solution C	K1048	24% w/w PEG 400, 18% TR-40 by volume in water.	32 mL	

Bulk components (5 and 20 mg)				
Component	**Cat. no.**	**Description**	**Quantity**	**Storage**
CCF4-AM	K1028	Dried down under vacuum from acetonitrile.	5 mg	–30°C to –10°C; Desiccate and protect from light.
CCF2-AM	K1023			
CCF4-AM	K1029		20 mg	

Loading solutions (Cat no. K1026, for 5 mg substrate)				
Component	**Part. no.**	**Description**	**Quantity**	**Storage**
DMSO for Solution A	K1035	—	10 mL	–30°C to –10°C; Desiccate and protect from light.
Solution B	K1036	100 mg/mL Pluronic®-F127 surfactant in DMSO and 0.1% acetic acid.	75 mL	
Solution C	K1037	24% w/w PEG 400, 18% TR-40 by volume in water.	800 mL	

Required materials and equipment

- Black-wall, clear-bottom, assay plates (with low fluorescence background), (Cat. no. 07-200-565, Thermo Fisher Scientific)
- Fluorescent plate reader capable of FRET using bottom read.
- Filters (see "Filter selection" on page 16)
- (*Optional*) Epifluorescence microscope, equipped with appropriate filters
- (*Optional*) Solution D, an anion transport inhibitor for cells such as CHO-K1 (Cat. nos. K1156 and K1157)

Important information

- The molecular weight of CCF2-AM is 1082.
- The molecular weight of CCF4-AM is 1096.
- Use the DMSO included in the kits to dissolve CCF2-AM or CCF4-AM when preparing Solution A.
- If Solution B is stored at temperatures cooler than 22°C, a white precipitate may form. This change does not affect the quality of the product. Warm and mix the solution at ~35°C until the precipitate dissolves, then use as described. Solution B which has been frozen solid cannot be restored.

Methods

Procedure guidelines

This general protocol is designed for loading cells with CCF2-AM or CCF4-AM before visual observation or analysis in a fluorescence plate reader. Use tissue culture-treated, black-wall, clear-bottom plates for this assay.

Note: See "Assay considerations" on page 15 for critical information necessary for a successful assay. Before proceeding with the assay for the first time, read this information carefully.

Controls

Include the following controls in each assay:

Control	Description
Positive Control	Cells should be stimulated with a known stimulant for the particular assay to insure that a detectable signal is obtained. Cells should have a significantly higher blue/green fluorescence ratio than the Negative Control. Under a fluorescent microscope, most Positive Control cells will appear blue. Refer to "(Optional) Visual Observation of intracellular CCF2-AM or CCF4-AM fluorescence" on page 18 for set up.
Negative control	Used to determine the amount of blue and green fluorescence to expect in an unstimulated sample. This value will also be used when determining the Response Ratio of your assay. Under a fluorescent microscope, most Negative Control cells will appear green. Refer to "(Optional) Visual Observation of intracellular CCF2-AM or CCF4-AM fluorescence" on page 18 for set up.
No Cells Background Control	Used to determine the amount of blue and green background fluorescence that exists in a cell-free sample. Subtract the amount of background fluorescence seen in the blue and green channels from the experimental wells containing cells to obtain accurate data. The signal in the green channel of unstimulated cells should be 10X greater than the cell-free sample signal.

Plate incubation

1. After preparing your plate of cells, incubate at 37°C/5% CO_2 for an amount of time sufficient for stimulation.
2. At the end of the incubation, allow the plate to equilibrate to room temperature before proceeding to "Incubation" on page 13.

Prepare Solution A

Solution A is a 1 mM stock solution of CCF2-AM or CCF4-AM in DMSO.

1. Prepare solution A according to the following table:

Amount of fluorescent substrate	Volume of DMSO	
	CCF2-AM	CCF4-AM
200 µg	185 µL	182 µL
5 mg	4.6 mL	4.6 mL
20 mg	18.5 mL	18.2 mL

2. Mix well.

   Note: Store the solution at –20°C, desiccated and protected from light. Aliquots of the 200 µg size are not required; you can aliquot the larger sizes for storage. Before each use, allow the frozen stock solution to thaw at room temperature and remove the desired amount of reagent. To reduce moisture uptake, re-cap the vial immediately after each use and return it to the desiccator in the –20°C freezer. Stored under these conditions, Solution A is stable for at least three months. Once thawed, Solution A may appear slightly yellow. This color change does not affect the quality or function of the product.

Choose a Substrate Loading Solution

We recommend using the Standard Loading Solution for most cells and the Alternative Loading Solution for cells such as CHO-K1 that load or retain the substrate poorly.

Standard Substrate Loading Solution

1. Add 6 µL of Solution A to 60 µL of Solution B and vortex.
2. Add 934 µL of Solution C to the combined Solutions A and B and vortex.

   Note: Under typical laboratory conditions, the 6X CCF2-AM Substrate Loading Solution is stable for up to 4 hours. 6X CCF4-AM Substrate Loading Solution is stable for up to 12 hours.

Alternative Substrate Loading Solution

1. Add 12 µL of Solution A to 60 µL of Solution B and vortex.
2. Add 898–853 µL of Solution C to the combined Solutions A and B and vortex.

   Note: The volume of Solution C is dependent on the volume of Solution D used in step 3 of this procedure.

   The final volume of Solution A + Solution B + Solution C + Solution D = 1000 µL.

3. Add 30 to 75 µL of the Solution D to the 6X Substrate Loading Solution and vortex.

   Note: Solution D is an anion transport inhibitor for cells such as CHO-K1.

Load cells with substrate

1. Add 6X Substrate Loading Solution to cells to 1X final concentration (for exampe, add 20 µL of 6X CCF2-AM or CCF4-AM Substrate Loading Solution to 100 µL of cells in buffer).
2. Add the same volume of 6X Substrate Loading Solution to the No Cells Background Control wells (containing assay medium or buffer) to 1X final concentration.

Incubation

1. Cover the plate to protect it from light and evaporation.
2. Incubate the plate at room temperature for 60–120 minutes, see "Load cells with substrate: guidelines" on page 15 for more information.

   Note: During the incubation, the cells will settle to the bottom of each well. Handle the plate gently as the cells must remain at the bottom of each well for accurate detection to occur.

Plate-reader detection

See "Plate-reader detection guidelines" on page 15 for guidelines on the visual observation of your plate.

1. Remove dust from the bottom of the plate with compressed air.
2. Be sure the plate reader is set to bottom-read mode.
3. Select the appropriate filters to allow excitation of the coumarin (~410 nm) and detection of the blue coumarin emission (~450 nm) and green fluorescein emission (~520 nm).
4. Read the plate.

Note: The correct filters are essential for a successful assay. For more information see "Filter selection" on page 16. For instrument-specific setup documents, go to **www.lifetechnologies.com/instrumentsetup**.

Analyze data

- Using the blue to green ratio to analyze samples reduces any well-to-well variations and provides for more consistent results.
- Background subtraction for both channels (460 nm and 530 nm) is essential to obtain accurate data. You may subtract both channels automatically using software connected to the fluorescence plate reader or manually after each assay plate has been read.

1. Calculate the average emission for the No Cells Background Control for the blue (~450 nm) and green (~ 520 nm) channels.
   This is your **average blue background** and **average green background**.

2. Subtract the *average blue background* from all of your controls and sample blue emissions. Subtract the *average green background* from all of your controls and sample green emissions.
   This is your **net blue signal** and **net green signal** for each well.

3. Well by well, calculate the ratio of blue to green fluorescence by dividing the *net blue signal* by the *net green signal*.
   This is your **blue to green ratio** for each well.

4. Determine the average *blue to green ratio* for your Negative Controls.
   This is your **average negative ratio**.

5. Calculate the **Response Ratio** for your Positive Control and experimental samples by dividing their *blue to green ratio* by the *average negative ratio*.
   This calculation normalizes the data so that the negative control wells have a mean value of 1.0. Due to the error-correcting nature of ratiometric readouts, subtle differences in Response Ratios with β-lactamase are more likely to be statistically significant.

6. *(Optional)* Use 8 replicates each of the Positive and Negative Control wells to calculate the Z' (Z-Factor) of your assay according to the following equation:

$$Z' = 1 - \frac{3\sigma_{stim} + 3\sigma_{unstim}}{|mean_{stim} - mean_{unstim}|}$$

Where	σ_{stim}	is the	standard deviation of experimental samples
	σ_{unstim}	is the	standard deviation of Negative Controls
	$mean_{stim}$	is the	mean of experimental samples
	$mean_{unstim}$	is the	mean of Negative Controls

Use the Z-factor to evaluate the quality of your experiment (Zhang et al., 1999).

Supplemental information

Assay considerations

Several variables may affect assay performance and will need to be determined empirically. Use the following suggestions as starting points; further evaluation may be necessary to optimize individual assay performance.

Cell stimulation

- Factors, such as the type of cell being tested and the necessary conditions for induction, can affect the optimal stimulation conditions for cells.
- Better results may be obtained with certain adherent cell types if the cells are adhered before stimulation.
- Certain assays may require the cells to be incubated in serum-free media for up to 24 hours before stimulation (serum-starvation).
- Alternative assay media formulations can help improve the response of some assays. Using serum that has been stripped of some components (including charcoal-dextran-treated and delipidated serum) is helpful in certain assays.

Load cells with substrate: guidelines

- Adherent cells are loaded at the density recommended in the cell line protocol.
- Suspension cells are typically loaded at $1 \times 10^6 – 2 \times 10^6$ cells/mL.
- The loading efficiency for adherent cell is density-dependent. Loading is most efficient when adherent cells are 60–80% confluent; completely confluent cells may load CCF2-AM or CCF4-AM much less efficiently.
- Different cell types load the substrate at different rates. To optimize substrate loading time, we recommend following the cell-line specific protocol or reading the plate every 30 minutes for 2 hours the first time an assay is run.

  Note: For increased sensitivity, you can extend the substrate loading time to a maximum of 3.5 hours (depending on the cell type).
- Some cell types exhibit poor substrate retention. For these cell types, we recommend using the Alternative Substrate Loading Solution (see "Alternative Substrate Loading Solution" on page 12).
- The loading rate is temperature-sensitive. Increasing the temperature (to 37°C, for example) will increase the loading rate, but actually results in a lower overall steady-state uptake of CCF2-AM or CCF4-AM because the export rate of CCF2-AM or CCF4-AM also increases. We recommend loading at room temperature.

Plate-reader detection guidelines

All measurements are made at room temperature from the bottom of the wells, preferably in black-wall, clear-bottom assay plates with low fluorescence background. Before reading the plate, remove dust from the bottom with compressed air.

Note: Some plates/fluorescence plate readers experience edge effects which may affect data. If edge effects are noticed, the plate layout should be considered when setting up the assay.

Instrumentation

Step by step instrument set-up guides are available at **www.lifetechnologies.com/instrumentsetup**. You can reach the specialized technical group directly at **drugdiscoverytech@lifetech.com**.

Filter selection

If your fluorescent plate reader does not include the appropriate filters, we recommend using the following filters available from Chroma Technologies (**www.chroma.com**).

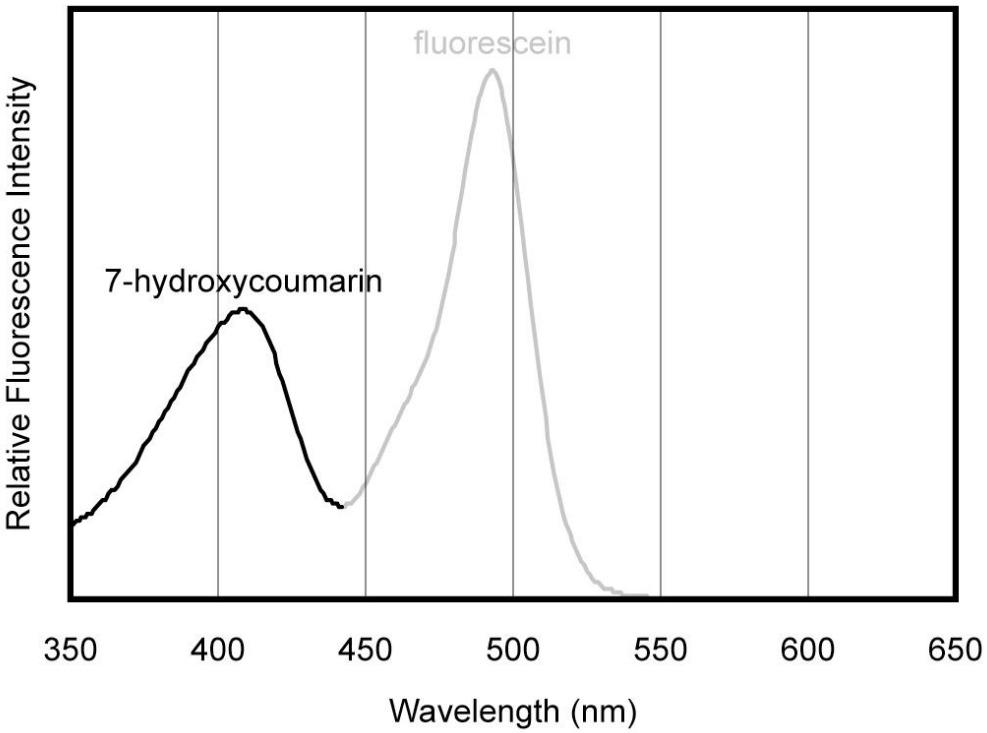
Table 1 Chroma Filters

Excitation filter	405 ± 10 nm/20x
Emission filter	460 ± 20 nm/40m
Emission filter	530 ± 15 nm/30m
Dichroic Mirror	425DCXR

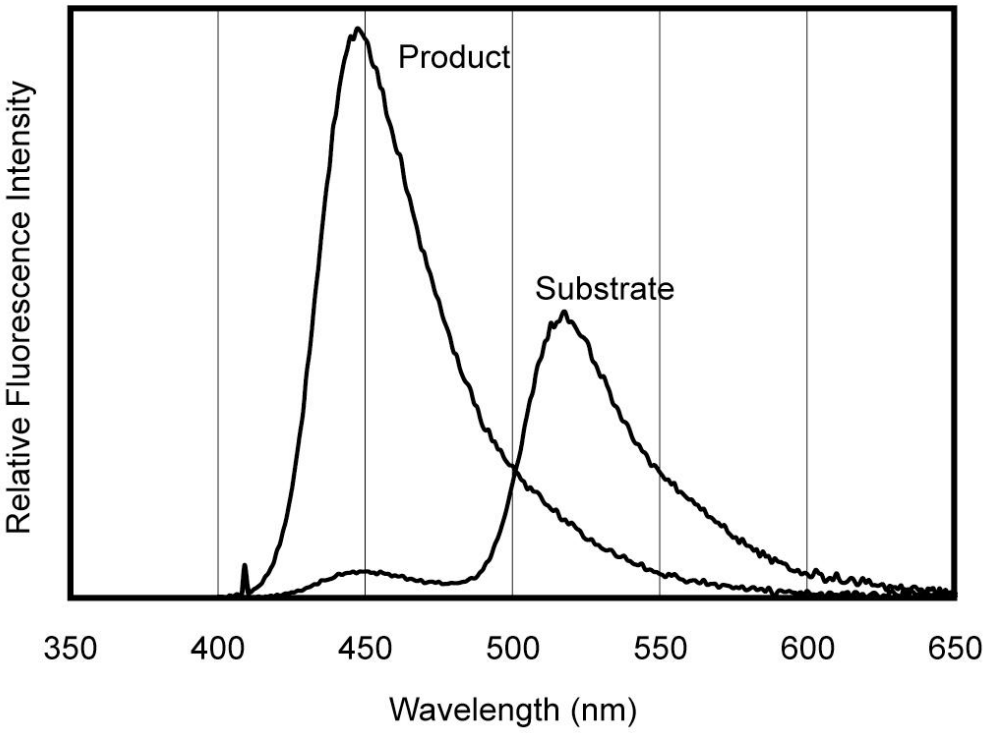
You may select alternative filters appropriate for CCF2 or CCF4 by using the spectra shown on the following page.

Note: To allow for FRET and ensure proper assay performance, the substrate must be excited at the 7-hydroxycourmarin wavelengths and not the fluorescein wavelengths.

Fluorescence Excitation Spectrum of CCF2-AM Substrate



Fluorescence Emission Spectra of CCF2-AM Substrate and CCF2 Product (excitation 409 nm)



(Optional) Visual Observation of intracellular CCF2-AM or CCF4-AM fluorescence

Photobleaching may occur during visual observation of the cells with a microscope. Therefore, if you plan to analyze the cells using a fluorescent plate reader, we recommend reading the plate before using the microscope.

- An inverted microscope equipped for epifluorescence and either a xenon or mercury excitation lamp is typically required to view the substrate fluorescence signal in cells.
- To visually inspect the cells, use a long-pass filter passing blue and green fluorescence light, so that your eye can visually identify whether the cells are fluorescing green or blue.
- For epifluorescence microscopes, a long-pass dichroic mirror is needed to separate excitation and emission light and should be matched to the excitation filter (to maximally block the excitation light around 405 nm, yet allow good transmission of the emitted light).
- We recommend using the filter set listed in the following table (available from Chroma Technologies [**www.chroma.com**]) for observing β-lactamase activity.

Table 2 Chroma filter set, Cat. no. 19011

Excitation filter	AT405/30x
Dichroic mirror	AT440DC
Emission filter	AT450lp

Filter sizes vary for specific microscopes and need to be specified when the filters are ordered. Go to **www.lifetechnologies.com/instrumentsetup** for instrument setup support.

B

Safety

WARNING! GENERAL SAFETY. Using this product in a manner not specified in the user documentation may result in personal injury or damage to the instrument or device. Ensure that anyone using this product has received instructions in general safety practices for laboratories and the safety information provided in this document.

- Before using an instrument or device, read and understand the safety information provided in the user documentation provided by the manufacturer of the instrument or device.
- Before handling chemicals, read and understand all applicable Safety Data Sheets (SDSs) and use appropriate personal protective equipment (gloves, gowns, eye protection, etc). To obtain SDSs, see the "Documentation and Support" section in this document.

Chemical safety

WARNING! GENERAL CHEMICAL HANDLING. To minimize hazards, ensure laboratory personnel read and practice the general safety guidelines for chemical usage, storage, and waste provided below, and consult the relevant SDS for specific precautions and instructions:

- Read and understand the Safety Data Sheets (SDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. To obtain SDSs, see the "Documentation and Support" section in this document.
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing).
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood).
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's cleanup procedures as recommended in the SDS.
- Handle chemical wastes in a fume hood.
- Ensure use of primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)
- After emptying a waste container, seal it with the cap provided.
- Characterize (by analysis if necessary) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure that the waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.
- **IMPORTANT!** Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.

Biological hazard safety

WARNING! BIOHAZARD. Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. All work should be conducted in properly equipped facilities using the appropriate safety equipment (for example, physical containment devices). Safety equipment also may include items for personal protection, such as gloves, coats, gowns, shoe covers, boots, respirators, face shields, safety glasses, or goggles. Individuals should be trained according to applicable regulatory and company/ institution requirements before working with potentially biohazardous materials. Follow all applicable local, state/provincial, and/or national regulations. The following references provide general guidelines when handling biological samples in laboratory environment.

- U.S. Department of Health and Human Services, *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, 5th Edition, HHS Publication No. (CDC) 21-1112, Revised December 2009; found at:
  www.cdc.gov/biosafety/publications/bmbl5/BMBL.pdf
- World Health Organization, *Laboratory Biosafety Manual*, 3rd Edition, WHO/CDS/CSR/LYO/2004.11; found at:
  www.who.int/csr/resources/publications/biosafety/Biosafety7.pdf

Documentation and support

Obtaining SDSs

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

Note: For the SDSs of chemicals not distributed by Thermo Fisher Scientific, contact the chemical manufacturer.

Obtaining Certificates of Analysis

The Certificate of Analysis provides detailed quality control and product qualification information for each product. Certificates of Analysis are available on our website. Go to **www.lifetechnologies.com/support** and search for the Certificate of Analysis by product lot number, which is printed on the box.

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)
- Search for user documents, 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

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at **www.lifetechnologies.com/termsandconditions**. If you have any questions, please contact Life Technologies at **www.lifetechnologies.com/support**.

References

Zhang, J-H., Chung, T. D. Y., and Oldenburg, K. R. 1999. A Simple Statistical Parameter for Use in Evaluation and Validation of High Throughput Screening Assays. *J Biomol Screen*. 4:67–73.