Instructions for use

sphingotest® Lightning

For the generation of luminescence signals in sphingotest® immunoassays.

For Research Use Only. Not for Diagnostic Procedures.

English

REF 080-04000/01

RUO

2...8°C

3000

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Intended use

The sphingotest® Lightning is a reagent intended for quantitative automated chemiluminescence measurement of sphingotest® immunoluminometric assays (ILMA) using acridinium ester technology in conjunction with an automated flash-type luminometer. The sphingotest® Lightning is intended for use by professional users in laboratory settings.

The product is for research use only. It is not intended for diagnostic or therapeutic purpose.

Background information

Reagents composed of hydrogen peroxide in nitric acid and sodium hydroxide are used to trigger the chemiluminescence of acridinium-ester in an oxidative reaction. In immunoassays, acridinium-esters are usually labelled to the secondary antibody also called tracer antibody (1).

This technology was developed in the 1980s and has been incrementally improved until the commonly used 2-step approach in which hydrogen peroxide in a nitric acid solution and sodium hydroxide are added sequentially to increase the light yield (2). The sphingotest® Lightning Initiators 1 and 2 are based on the described technology.

Test principle

The recording of the luminescence signal that is released within a few seconds is performed in flash-type microtiter plate (MTP) luminometers (3).

sphingotest® Lightning reagents (Initiator 1 and 2) are especially designed and optimized for the use with sphingotest® immunoluminometric assays that utilize acridinium-ester technology. The reagents are ready to use and are connected to a flash-type MTP luminometer for easy and error reduced handling. The hydrogen peroxide in the Initiator 1 reacts with acridinium esters coupled to an antibody in an acidic reaction mix to first convert all active pseudo-bases – without luminescent potential – to the active ester molecules. Initiator 2 contains sodium hydroxide and initiates the light emitting reaction upon a fast increase of pH. The reaction emits an intense light flash around 440 nm (4), which is detected by the flash-type MTP luminometer.

The sphingotest® Lightning controls provided are used to perform a “Light Inspection Check”. They allow to verify system suitability of the used luminometer connected with the sphingotest® Lightning Initiators and provide information on signal dynamics between the Non-Specific Background (NSB) and a low signal (tare dynamic) as well as between a high and a low signal (net dynamic) using both Control Low and High (5).

Reagents and materials provided

Material	**Quantity**	**Description and Reconstitution**
LIGI1 LIGI2	2 x 3 bottles, 110 mL each, liquid	3 bottles per each Initiator, ready to use.
CONL CONH	2 x 2 vials, lyophilised	Lyophilised Lightning-Control Low (CONL) and Control High (CONH). to be reconstituted with 2 mL ultra-pure water.
IFU	1	Print version of the instructions for use.
Quality report	1	Lot specific quality report.

Reagents and materials required but not provided

- F8 Strip Plate (microplate with 12 strips and each 8 wells, uncoated, white, F-bottom); for running the Light Inspection Check using the sphingotest® Lightning Controls Low and High.

- Microtiter pipette (25 µL) with exchangeable plastic tip
- Vortex mixer
- Ultra-pure water

Special material required but not provided

- Flash-type MTP luminometer with two injectors, capable to inject each 100 µL injection volume (e.g., Centro LB 960 or Centro LB 963, Berthold Technologies GmbH & Co. KG, Wildbad, Germany)
- Software and validation tool compatible with the MTP luminometer (e.g., Instrument Control and Evaluation, Berthold Technologies GmbH & Co. KG, Wildbad, Germany)

Information on device(s) and equipment to combine with sphingotest® Lightning

The sphingotest® Lightning is used as accessory for the sphingotest® immunoassays in combination with an automated flash-type MTP luminometer at ambient temperature. For further details concerning the operation of the luminometer, please refer to the manufacturer's instructions for use.

The software used with the MTP luminometer must be programmed with the following specifications to ensure proper measurement of the luminescence signals:

#	Step	Specification
1	Delay	120 seconds
2	Injection of LIGI1 into well before measurement position	100 µL
3	Injection of LIGI2 into well in measurement position	100 µL
4	Measurement duration	1 second

Delay is set for the whole strip plate when inserted into the luminometer.

Ensure that injection and measurement is done together per well (steps 2 to 4) and that there is no significant delay between the injections of Initiator 1 and Initiator 2 and no set delay between the injection of Initiator 2 and measurement of the flash luminescence signals. The injection speed should be set to low (200 µL/sec) to prevent the reagent liquid from spilling over.

Key performance characteristics of device(s) and equipment to combine with sphingotest® Lightning

The flash-type MTP luminometer used in conjunction with the sphingotest® Lightning must have the minimum specifications as given in the table below:

Parameter	Specification
MTP type	96-well, flat-bottom, white
MTP dimension (LxWxH)	128.2 x 86.0 x 14.77 mm
Detection unit	Photomultiplier with a spectral range of 340–639 nm
Detection principle	Flash luminescence
Detection sensitivity	< 10 amol ATP
Crosstalk	$< 10^{-6}$
Dynamic range	> 6 decades
Number of injectors	2
Injection volume	100 µL

The acquisition software and validation tool must be compatible with the used luminometer.

Restrictions

The sphingotest® Lightning has been validated for the quantitative measurement of the sphingotest® immunoassays with Centro LB 960 and Centro LB 963 (Berthold Technologies GmbH and Co KG, Wildbad Germany). ICE software (Berthold Technologies GmbH and Co KG, Wildbad Germany) and MikroWin 2010 software (Labsis Laborsysteme GmbH, Neunkirchen-Seelscheid, Germany) were used for instrument control and evaluation.

If another type of MTP luminometer and/or software or other immunoassays are used, the user should validate the performance of acquiring the luminescence signals and calculating the results.

Storage and handling of reagents

The unopened kit must be stored at 2...8°C in the kit packaging until usage. The expiry date specified on the kit packaging and reagents must be observed under all circumstances.

Opened reagents (Initiator 1 and 2) are stable for up to 30 days if kept at 18…25 °C (in-use stability).

It is recommended to disconnect the reagents from the luminometer after completion of daily measurements with storage at ambient condition until next use.

Reconstituted controls are stable for 30 days if stored at 2…8 °C (in-use stability).

Warnings and precautions

- For research use only. Not for diagnostic procedures.
- The product must only be used by professional users in a laboratory setting.
- Store the unopened kit at 2...8°C. Do not use kit components after the expiry date has passed.
- Read the instructions for use carefully before use. Performance can be affected if reagents are improperly handled or stored under conditions other than those indicated in this IFU.
- Do not freeze any parts of the kit.
- Do not use damaged kits.
- Use only components belonging to the same kit. Do not combine reagents from kits with different lot numbers or other manufacturers.
- Wear protective clothing such as laboratory coats, eye/face protection and disposable gloves whenever kit components and human specimens are handled.
- The sphingotest® Lightning reagent LIGI2 contains sodium hydroxide and
  - may be corrosive to metals,
  - causes skin irritation,
  - causes serious eye irritation.
- In case of skin contact: Wash with plenty of soap and water. If skin irritation or rash occurs: Get medical advice/attention.
- In case of eye contact: Irrigate copiously with clean, fresh water for at least 15 minutes, holding the eyelids apart. Remove contact lenses, if present and easy to do. Continue rinsing. If eye irritation persists: Get medical advice/attention.
- The controls contain material of animal origin.
- Bring the reagents and controls to room temperature before use.
- Carefully close all containers after use.
- As glass bottles are used for controls, we specifically point out the possible hazard of breakage and thereby the associated hazard of injury.
- sphingotest® Lightning was evaluated with Centro LB 960 and Centro LB 963 (Berthold Technologies GmbH & Co. KG, Wildbad, Germany). Before using any other equipment, a priori assessment by the user is required.
- Due to the formulation of the reagents the injection system may become harmed in case reagents are connected for longer times. It is recommended to disconnect the reagents from the luminometer after completion of daily measurements and to rigorously rinse the injection system (injectors / pumps and tubes) with ultra-pure water.

- The disposal of reagents as well as waste materials originating from the test preparations must be performed under observation of the local regulations for laboratory waste.

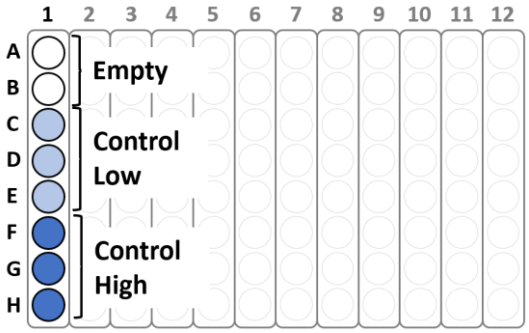
Preparations for luminescence measurement

1. Reagent preparation: Allow unopened reagents (Initiator 1 and 2) to equilibrate to room temperature for at least one hour.
2. Remove all other reagents or solution from the injection system (injectors, pumps and tubes) before connecting the sphingotest® Lightning Initiator 1 and 2. For the emptying and rinsing of the injection systems refer to information provided in the operating instructions of the used luminometer.
3. Connect the sphingotest® Lightning measuring reagents Initiator 1 and Initiator 2 to the injection system. When assigning the measuring reagents to the injection system, special attention must be given that the addition of Initiator 1 is performed before addition of Initiator 2 (reagent for the triggering of the luminescence reaction).
4. Fill and rinse the injection systems (injectors, pumps and tubes) with proper volume of the connected Initiator 1 and 2 before starting measurements. For filling and rinsing information refer to the operating instructions of the luminometer used.
5. The luminometer is now set up for measurements.

Quality control procedure – Light Inspection Check

It is recommended to use the sphingotest® Control Low and High in combination with a F8 strip from a strip plate (12 x 8, uncoated, white, F-bottom) for a daily system suitability test of the used MTP flash luminometer.

1. Preparation of controls:
   a. Allow unopened controls (Low and High) to equilibrate to room temperature for 10 minutes.
   b. Reconstitute each with 2 mL of ultra-pure water. Homogenize the solution by gentle mixing and make sure that all lyophilised material is resolved. Skip this step, in case using already reconstituted controls.
2. Use a strip plate frame containing one F8 strip (not provided, see section special material needed but not provided) for sampling.
3. Pipette 25 µL of Control Low to wells C to E (triplicate measurements) and 25 µL of Control High to wells G to H (triplicate measurement). Note: The first two wells (A and B) remain empty for measurement of the Non-Specific Background (NSB) (duplicate measurement). See also pipetting scheme below:



4. Insert the strip plate to the prepared luminometer and measure luminescence signals using the specifications described under “Information on device(s) and equipment to combine with sphingotest® Lightning”.
5. Calculate the tare dynamic by dividing the obtained mean signal for Control Low by the obtained mean signal from the empty wells (Non-Specific Background (NSB)).

$$\textit{Tare Dynamic} = \frac{[Signal\,(Control\ Low)]}{[NSB]}$$

6. Calculate the net dynamic by dividing the obtained mean signal for Control High by the obtained mean signal for Control Low.

$$Net\ Dynamics\ = \frac{[Signal(Control\ High) - NSB]}{[Signal(Control\ Low)\ - NSB]}$$

7. Store opened controls at 2…8°C until next usage.

Interpretation of results

The determined values for tare dynamic and net dynamic are compared to the target values and ranges stated in the provided lot-specific quality report (QR). The coefficient of variation (CV%) for the measured mean signals should not exceed 10% for Control Low and High.

Please note hereby that the target values and ranges represented in the QR has been defined with using a Centro LB 960 luminometer (Berthold Technologies GmbH & Co. KG, Wildbad, Germany).

Trouble Shooting

In the event that the determined Light Inspection Check results deviate from those of the quality report (QR), the following error sources/causes and solutions are possible:

1. Result:

   Non-Specific Background (NSB) elevated, tare dynamic is smaller than target value.

   Possible cause:

   Contamination of the sphingotest® Lightning measuring reagents (Initiator 1 and/or 2) or the injection system (pumps/injectors and/or tubes with luminogens (e.g., luminescence tracer).

   Possible solution:

   a. Use of new sphingotest® Lightning measuring reagents or
   b. use of new tubes or
   c. intensive rinsing of both injection systems with a 1:1 mixture of both sphingotest® Lightning measuring reagents.

2. Result:

   Non-Specific Background (NSB) very low, Tare and Net Dynamics roughly 1.

   Possible Cause:

   One of the sphingotest® Lightning measuring reagents (Initiator 1 or 2) not present or unusable.

   Possible solution:

   a. Verification of the injection systems or
   b. use of new sphingotest® Lightning measuring reagents.

3. Result:

   Tare and Net Dynamics smaller than target value and outside of the target range.

   Possible cause:

   One of the sphingotest® Lightning measuring reagents (Initiator 1 or 2) is unusable.

   Possible solution:

   Use of new sphingotest® Lightning measuring reagents.

4. Result:

   Coefficient of variation (CV) of the replicates >10%.

   Possible cause:

   Injection systems of the luminometer work unevenly, or faulty pipetting of the concerned sphingotest® Lightning control.

   Possible solution:

   a. Verification of the pipette used for pipetting the sphingotest® Lightning controls, and/or
   b. verification of the injection systems for possibly present air bubbles, and/or

c. verification of the injection systems by means of suitable methods regarding correctness and reproducibility of the injected measuring reagent volumes (each injection 150 µL for microtiter plate luminometer), and/or

d. verification of the filters connected to the tubes of the injection systems for luminogen contamination.

Limitations

Unreliable results may occur due to deviations from the procedure described in the instructions for use.

Notice to the user

Please immediately report any serious incident in relation to this assay to the relevant competent authority in your country and SphingoTec GmbH.

For getting the appropriate Safety Data Sheets according to EC directive 1907/2006 (REACH) or technical assistance and more information please contact our distribution partner or Sphingotec GmbH (see contact information on the kit label).

Literature

1. Weeks I, Beheshti I, McCapra F, Campbell AK, Woodhead JS. Acridinium esters as high-specific-activity labels in immunoassay. Clin Chem. 1983;29(8):1474-9.
2. Ronald A, Stimson WH. Evolution of immunoassay technology. Parasitology. 1998 117(7):13-27.
3. Dyke van K, McCapra F, Behesti I. Bioluminescence and Chemiluminescence Instruments and Applications: CRC Press; 1985. 1-42 p.
4. Dodeigne C, Thunus L, Lejeune R. Chemiluminescence as diagnostic tool. A review. Talanta. 2000;51(2000): 415–39
5. Gadow A, Hantke U. Improved Reagent Kit for Optimal Generation of Diacyl-Hydrazide Chemiluminescence and Quality-Control Assessment of the System. in Bioluminescence and Chemiluminescence. Proceedings of the 4th International Bioluminescence and Chemiluminescence Symposium 1986.

Symbols

Symbol	Application	Symbol	Application	Symbol	Application
	Consult instructions for use		Article Number.		Do not re-use
	For Research use only. Not for diagnostic procedures.		Contents sufficient for (number of) single determinations		Use by date
	Temperature limit		Batch code		GHS05 -Corrosive
	Green dot according to German legislation		Manufacturer		Distributor
	Reconstitution		Lyophilised		

sphingotest® is a registered trademark. Other product names used in this document are for identification purposes and may be brands and/or trademarks of their respective companies.

Document Revision History

Revision No.	Date	Changes
01	September 2022	Initial release