

Reportasol™ Extraction Buffer



Description

Reportasol Extraction Buffer	25 ml	70909-3
	125 ml	70909-4

Reportasol Extraction Buffer is a buffer formulated for efficient mammalian cell lysis and preservation of extremely high reporter enzyme activity. A proprietary mixture of non-ionic and zwitterionic detergents, Reportasol disrupts the plasma membrane, leading to release of soluble reporter enzymes without need for freeze/thaw, fractionation, sonication, or other secondary treatments. Reportasol has been specifically formulated for optimal activity of firefly luciferase, *Renilla* luciferase, and β -galactosidase reporters, and is also compatible with BCA protein assays.

Storage

Reportasol Extraction Buffer should be stored at -20°C .

Using Reportasol Extraction Buffer

Reportasol should be thawed just prior to use. If small quantities of Reportasol are needed for an experiment, the reagent should be dispensed into small aliquots and stored at -20°C . Repeated freeze/thawing of Reportasol may lead to decreased activity. Reportasol works in passive mode. No scraping or vortexing of cells is required, although additional activity may be extracted by gentle agitation, scraping or vortexing. Furthermore, since the extraction methodology is gentle, a clarification centrifugation step is not generally required.

Table 1: Recommended volumes of Reportasol for extraction

Culture Format	Surface Area (cm ²)	Volume of Reportasol
96 Well Plate	0.32	30 μl
48 Well Plate	0.8	50 μl
24 Well Plate	2.0	100 μl
12 Well Plate	4.0	200 μl
6 Well Plate	9.6	300 μl
35 mm Dish	9.6	300 μl
60 mm Dish	21.0	500 μl
100 mm Dish	55.0	1.0 ml
T-25 Flask	25.0	500 μl
T-75 Flask	75.0	1.5 ml
Suspension cells	10 ⁶ cells*	150 μl

*Suspension cells vary greatly in cell size; thus adjustment may be necessary.

Extraction of monolayer cultures

1. Thaw Reportasol and keep on ice until use. The Reportasol bottle may be placed in a room temperature water bath to speed thawing.
2. Aspirate culture medium from cells.
3. **Optional:** If components of the culture media (i.e. phenol red) are inhibitory to reporter enzyme analysis, wash cells once with PBS (PBS; 43 mM Na₂HPO₄, 15 mM KH₂PO₄, 137 mM NaCl, 27 mM KCl, pH 7.4) or Hanks' Buffered Salts Solution (HBSS) prior to Reportasol addition.
4. Add the recommended volume of ice-cold Reportasol to each well or flask (refer to Table 1 above) and incubate at room temperature for 5 min.
5. Remove extract to a suitably sized tube and begin reporter assays. Cells plated in 96 well format can be assayed directly in the plate for reporter enzyme activity.

Note: In general, extract clarification is not required. However, in cases of very high cell density, or if active manipulation of cells has been employed (agitation, vortexing, or scraping), a centrifugation step may be required to clarify the extract. A 5 min centrifugation at top speed in a microcentrifuge is sufficient ($\sim 15,000 \times g$, 4°C), followed by transfer of the supernatant to a new tube.

Copyright © 2000 by Novagen, Inc. All rights reserved. Reportasol and the Novagen name and logo are trademarks and registered trademarks of Novagen, Inc.

United States & Canada 800-207-0144
Germany 0800 6931 000
United Kingdom 0800 622935
Or your local sales office

TB301 02/01

Novagen

1



Reportasol™ Extraction Buffer

Extraction of suspension cells

1. Pellet the cells by low speed centrifugation (e.g., 5 min at 2500 × g).
Optional: If components of the culture media (i.e. phenol red) are inhibitory to reporter enzyme analysis, wash cells once with PBS or HBSS prior to Reportasol addition. Pellet cells as above and discard supernatant.
2. Resuspend the cells in the recommended volume of ice-cold Reportasol (Table 1 above).
3. Incubate at room temperature for 5 min.
4. Clarify the extract by centrifugation at 15,000 × g for 5 min (4° C).

Note: A centrifugation step is required to clarify the extract when working with suspension cells.

5. Transfer supernatant to a fresh tube and begin analysis.

Related products

Product	Size	Cat. No.
Reporter Assay	500 assays	70979-3
BetaFluor™ β-Galactosidase Assay Kit	2500 assays	70979-4
BetaRed™ β-Galactosidase Assay Kit	500 assays	70978-3
	2500 assays	70978-4
FRETWorks™ S•Tag Assay Kit	100 assays	70724-3
	1000 assays	70724-4
S•Tag™ Rapid Assay Kit	100 assays	69212-3
CytoBuster™ Protein Extraction Reagent	50 ml	71009-3
	250 ml	71009-4
pTriEx Multisystem Expression Vectors	see catalog	
Transfection and DNA Purification		
GeneJuice™ Transfection Reagent	1 ml	70967-3
	10 ml	70967-4
Mobius™ 200 Plasmid Kit	25 rxn	70970-3
Mobius 200 Columns	25 rxn	71019-3
Mobius 500 pET Plasmid Kit	10 rxn	70969-3
Introductory Mobius 1000 Plasmid Kit	2 rxn	70854-3
Mobius 1000 Plasmid Kit	10 rxn	70853-3
	25 rxn	70853-4
Introductory UltraMobius™ 1000 Plasmid Kit	2 rxn	70907-3
UltraMobius 1000 Plasmid Kit	10 rxn	70906-3
	25 rxn	70906-4
ClearSpin™ Filter	pkg/25	70848-3
Mobius Buffer Kit	10 rxn	70855-3
Mobius 1000 Columns	10 rxn	70849-3
	25 rxn	70849-4