Procarta™ Transcription Factor Whole Cell Lysis Kit

User Manual

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Panomics

Panomics, Inc.

Procarta Transcription Factor Whole Cell Lysis Kit User Manual

Citing Procarta in Publications

When describing a procedure for publication using this product, we would appreciate it if you would refer to it as the Procarta™ TF Whole Cell Lysis Kit.

If a paper cites a Procarta product and is published in a research journal, the lead author(s) may receive a travel stipend for use at a technology conference or tradeshow by sending a copy of the paper to our technical support group at techsupport@panomics.com or via fax at (510) 818-2610.

Disclaimer

Panomics, Inc. reserves the right to change its products and services at any time to incorporate technological developments. This manual is subject to change without notice.

Although this manual has been prepared with every precaution to ensure accuracy, Panomics, Inc. assumes no liability for any errors or omissions, nor for any damages resulting from the application or use of this information.

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About the User Manual

Who Should Read this Manual

Anyone that has purchased a Procarta Whole Cell Lysis Kit from Panomics to prepare whole cell lysates for use in Panomics' Procarta Transcription Factor (TF) Assay Kits.

What this Manual Covers

This manual provides the following:

- Kit contents
- Required materials and equipment
- Whole cell lysis procedure

Safety Warnings and Precautions

CAUTION All chemicals should be considered potentially hazardous. We recommend that this product and its components be handled by those trained in laboratory techniques and be used according to the principles of good laboratory practice.

Note This product is intended for research use only.

For More Information

For information about the Procarta products mentioned in this manual, visit our website at www.panomics.com.

Procarta Transcription Factor Whole Cell Lysis Assay Kit

About the Transcription Factor Whole Cell Lysis Kit

The Procarta TF Whole Cell Lysis Kit contains reagents and procedures for the preparation of whole cell lysates for use in our Procarta TF Assay Kits. The Procarta TF Whole Cell Lysis Kit contains sufficient reagents for the preparation of 40 whole cell lysates from cultured cells grown in 6-well culture plates or 240 whole cell lysates from cultured cells grown in 96-well culture plates.

Kit Contents and Storage

The Procarta Transcription Factor Whole Cell Lysis Assay Kit contains the following components.

Procarta TF Whole Cell Lysis Kit components:

Component	Quantity	Storage
Lysis Buffer I	12.0 mL	–20 °C
Lysis Buffer II	1.2 mL	–20 °C
DTT	120 µL	–20 °C
Protease inhibitor cocktail	120 µL	–20 °C

Required Materials and Equipment Not Provided

Materials and Equipment

Item	Source
1X PBS	Invitrogen (P/N 14190-144)
Rocking platform	VWR, Rocking Platform, Model #100 or equivalent
Centrifuge	Eppendorf #5804R
Protein determination kit	Bio-Rad DC Protein Assay Kit (P/N 500-0112) or equivalent
Microcentrifuge tubes	Major laboratory supplier (MLS)
15 mL conical centrifuge tubes	MLS
Adjustable single and multi-channel precision pipettes	MLS
PCR plates	MLS

Cell Preparation

Growing Cells

In all cases, cells are grown to about 90% confluence. The following table provides recommendations for the cell requirements for each culture vessel type. However, it is important to realize that cell types vary in size and actual numbers of cells/vessel may vary.

Use the table below as a guide.

Culture Vessel	Cell Number
100-mm culture dish	1 x 10^7 cells/dish
6-well plate	1–5 x 10^6 cells/well
24-well plate	2–5 x 10^5 cells/well
96-well plate	2–5 x 10^4 cells/well

Whole Cell Lysis Procedure

Assay Guidelines

IMPORTANT All components and PBS must be kept on ice at all times. Lysis Buffer 1 Working Reagent must be kept on ice and should be used within 2 hours of preparation.

Preparing Working Reagent

To prepare working reagent:

Step	Action
1	For preparation of 1 mL Lysis Buffer 1 Working Reagent, combine the following reagents, then invert tube to mix: 1 mL Lysis Buffer 1 10 µL DTT 10 µL Protease Inhibitor Cocktail Scale Lysis Buffer preparation according to experimental design. Use the table below as a guide.

Vessel	Quantity of Lysis Working Reagent
100 mm culture dish	1 mL/dish
6-well plate	250 µL/well
24-well plate	100 µL/well
96-well plate	50 µL/well

Preparing Whole Cell Lysates From Adherent Cells

To prepare working reagent:

<table>
<tr><th>Step</th><th>Action</th></tr>
<tr><td>1</td><td>Remove the culture media from all wells and wash cells twice with an appropriate volume of cold 1X PBS.

<table><tr><th>Vessel</th><th>Quantity of PBS</th></tr><tr><td>100 mm culture dish</td><td>10 mL/dish</td></tr><tr><td>6-well plate</td><td>1 mL/well</td></tr><tr><td>24-well plate</td><td>500 µL/well</td></tr><tr><td>96-well plate</td><td>200 µL/well</td></tr></table></td></tr>
<tr><td>2</td><td>Following the second wash, make sure the PBS is completely removed.</td></tr>
<tr><td>3</td><td>Add the appropriate volume of Lysis Buffer I Working Reagent to the wells.

<table><tr><th>Vessel</th><th>Quantity of Lysis Buffer I Working Reagent</th></tr><tr><td>100 mm culture dish</td><td>1 mL/dish</td></tr><tr><td>6-well plate</td><td>250 µL/well</td></tr><tr><td>24-well plate</td><td>100 µL/well</td></tr><tr><td>96-well plate</td><td>50 µL/well</td></tr></table></td></tr>
<tr><td>4</td><td>Transfer culture vessel(s) to an ice bucket and transfer ice bucket to a rocking platform at 200 rpm for 10 minutes.</td></tr>
<tr><td>5</td><td>Add the appropriate volume of Lysis Buffer II Working Reagent to the wells.

<table><tr><th>Vessel</th><th>Quantity of Lysis Buffer II Working Reagent</th></tr><tr><td>100 mm culture dish</td><td>100 µL/dish</td></tr><tr><td>6-well plate</td><td>25 µL/well</td></tr><tr><td>24-well plate</td><td>10 µL/well</td></tr><tr><td>96-well plate</td><td>5 µL/well</td></tr></table></td></tr>
<tr><td>6</td><td>Transfer culture vessel(s) to an ice bucket and transfer ice bucket to a rocking platform at 200 rpm for 1 hour.</td></tr>
<tr><td>7</td><td>Pipet up and down several times, then transfer each sample to a 1.5 mL microcentrifuge tube and centrifuge at 14,000 x g for 3 minutes at 4°C. Note the orientation of tube in centrifuge as pellets may not be visible.

For 96-well plates, pipet up and down several times, then transfer well contents from the cell culture plate to a PCR plate and centrifuge the PCR plate at 2,250 x g for 5 minutes.</td></tr>
<tr><td>8</td><td>Transfer supernatant(s) to new microcentrifuge tubes or a new PCR plate, this is your whole cell lysate.</td></tr>
</table>

To prepare working reagent: *(continued)*

Step	Action
9	Measure the protein concentration of each sample using a protein quantitation assay (sold separately). Then prepare 5 µL aliquots for each of the samples. Store samples at –80 °C or use immediately in Procarta TF Assay Kit. **Vessel** / **Typical Protein Yields** 100 mm culture dish: 300–800 µg/dish 6-well plate: 200–500 µg/well 24-well plate: 40–80 µg/well 96-well plate: 20–40 µg/well

Preparing Whole Cell Lysates From Suspension Cells

To prepare whole cell lysates from suspension cells:

Step	Action
1	Transfer cells to a 1.5 mL or 15 mL centrifuge tube as appropriate and centrifuge at 500 x g for 5 minutes. For 96-well plates, transfer cells to a PCR plate and centrifuge at 500 x g for 5 minutes
2	Remove the culture media and wash cells by resuspending in 1 mL of cold 1X PBS followed by centrifugation at 500 x g for 5 minutes. Repeat wash step. For 96-well plates, remove the culture media and wash cells by resuspending in 200 µL of cold 1X PBS followed by centrifugation at 500 x g for 5 minutes. Repeat wash step. Following the second wash step, ensure that the 1X PBS solution is completely removed from the cells. **Note** Before the second centrifugation, transfer contents from the 15 mL centrifuge tube to a 1.5 mL microcentrifuge tube.
3	Immediately add the appropriate volume of Lysis Buffer 1 Working Reagent to cell pellets. Mix by pipetting up and down several times. **Original Culture Vessel** / **Lysis Buffer I Working Reagent** 100 mm culture dish: 1 mL/dish 6-well plate: 250 µL/well 24-well plate: 100 µL/well 96-well plate: 50 µL/well
4	Transfer tube(s) or culture plate to an ice bucket and place on a rocking platform at 200 rpm for 10 minutes.

To prepare whole cell lysates from suspension cells: *(continued)*

<table>
<tr><th>Step</th><th>Action</th></tr>
<tr><td>5</td><td>Add the appropriate volume of Lysis Buffer II to the cells.
<table>
<tr><th>Original Culture Vessel</th><th>Quantity of Lysis Buffer II Working Reagent</th></tr>
<tr><td>100 mm culture dish</td><td>100 µL/dish</td></tr>
<tr><td>6-well plate</td><td>25 µL/well</td></tr>
<tr><td>24-well plate</td><td>10 µL/well</td></tr>
<tr><td>96-well plate</td><td>5 µL/well</td></tr>
</table></td></tr>
<tr><td>6</td><td>Transfer tube(s) or culture plate to an ice bucket and place on a rocking platform at 200 rpm for 1 hour.</td></tr>
<tr><td>7</td><td>For microcentrifuge tubes, pipet up and down several times and centrifuge at maximal speed (12,000 x g) for 3 minutes at 4°C. Note the orientation of tube in centrifuge as pellets may not be visible.

For PCR plates, pipet up and down several times and centrifuge the PCR plate at 2,250 x g for 5 minutes.</td></tr>
<tr><td>8</td><td>Transfer supernatant(s) to a new microcentrifuge tube or a new PCR plate, this is your whole cell lysate.</td></tr>
<tr><td>9</td><td>Measure the protein concentration of each sample using a protein quantitation assay (sold separately). Then prepare 5 µL aliquots for each of the samples. Store samples at –80 °C or use immediately in Procarta TF Assay Kit.
<table>
<tr><th>Vessel</th><th>Typical Protein Yield</th></tr>
<tr><td>100 mm culture dish</td><td>300–800 µg/dish</td></tr>
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<tr><td>24-well plate</td><td>40–80 µg/well</td></tr>
<tr><td>96-well plate</td><td>20–40 µg/well</td></tr>
</table></td></tr>
</table>

Contacting Panomics

Technical Help

For technical questions, contact our technical support group by telephone at 1-877-726-6642 option 3 or by email at techsupport@panomics.com (US and Canada) or techsupport_europe@panomics.com (Europe), or visit our website www.panomics.com for an updated list of FAQs and product support literature.

For Additional Services

For information about Panomics products or for ordering information, contact your Regional Sales Manager, or visit our website at www.panomics.com.