Protocol for Parallel Analysis of RNA and Protein Expression Using SingleShot™ Cell Culture Lysates

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This protocol is designed for cultured cells grown in tissue culture plates. Volumes indicated are for analysis of one entire tissue culture plate. For analysis of a partial plate, adjust volumes accordingly.

Materials

Note: Additional materials and equipment are needed to carry out the RT-qPCR and western blotting portions of this protocol. Refer to bulletin 6774 for more details.

- Cultured cells in 6-, 12-, 24-, 48-, or 96-well tissue culture plates
- SingleShot Cell Lysis RT-qPCR Kit (see Table 6 for recommended kits)
- 4x Laemmli Buffer (catalog #1610747)
- β-mercaptoethanol (catalog #1610710)
- 1x phosphate buffered saline (prepared from 10x PBS buffer, catalog #1610780)

Equipment

- T100™ Thermal Cycler (catalog #1861096)

Procedure

1 Preparation of RNA and Protein Lysis Buffers

1.1 Prepare the appropriate volume of RNA lysis buffer according to Table 1. Mix thoroughly and store on ice. Use within 2 hr of preparation.

Table 1. Preparation of RNA lysis buffer.

Component	6-Well	12-Well	24-Well	48-Well	96-Well
SingleShot Cell Lysis Buffer, µl	627	1,000	1,500	2,000	2,000
Proteinase K, µl	16.5	26.4	39.5	52.8	52.8
DNase I, µl	16.5	26.4	39.5	52.8	52.8
Total, µl	**660**	**1,053**	**1,579**	**2,105**	**2,105**

1.2 In a fume hood, prepare the appropriate volume of protein lysis buffer according to Table 2. Mix thoroughly and store on ice. Use within 2 hr of preparation.

Table 2. Preparation of protein lysis buffer.

Component	6-Well	12-Well	24-Well	48-Well	96-Well
4x Laemmli Buffer, µl	545	630	594	792	792
β-mercaptoethanol, µl	60.5	70	66	88	88
Total, µl	**606**	**700**	**660**	**880**	**880**

2 Cell Harvest and Lysate Preparation

2.1 Aspirate culture medium completely from cells and discard.

2.2 Wash cells gently with room temperature PBS buffer. Aspirate and discard PBS buffer.

2.3 To prepare cell lysates, add the appropriate amount of SingleShot Cell Lysis Buffer to each well according to Table 3. Rotate the plate to make sure that the lysis buffer covers the entire well. Do not mix by pipetting. Keep plate on ice for 5 min.

Table 3. Amount of cell lysis buffer to add to each well.

Component	6-Well	12-Well	24-Well	48-Well	96-Well
SingleShot Cell Lysis Buffer, µl	300	180	90	60	30

2.4 To prepare RNA lysates, dispense the amount of RNA lysis buffer indicated in Table 4 into the appropriate number of wells of a PCR plate. Add the indicated amount of cell lysate to the wells. Store on ice.

Table 4. Preparation of RNA lysate (per well)

Component	6-Well	12-Well	24-Well	48-Well	96-Well
RNA lysis buffer, µl	100	80	60	40	20
Cell lysate, µl	25	20	15	10	5
Total, µl	**125**	**100**	**75**	**50**	**25**

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2.5 In the T100 Thermal Cycler, incubate the RNA lysates according to the following protocol: 25°C for 10 min, 37°C for 5 min, and 75°C for 5 min. The RNA lysates are now ready for RT-qPCR analysis. They can be stored on ice for 4 hr, at –20°C for up to 1 month, or at –80°C for up to a year.

2.6 To prepare the protein lysates, add the indicated amount of protein lysis buffer (according to Table 5) to the remaining cell lysate. Mix by pipetting. The protein lysates are ready for western blot analysis. If not using immediately, transfer to microcentrifuge tubes and store at –80°C until needed.

Table 5. Preparation of protein lysate.

Component	6-Well	12-Well	24-Well	48-Well	96-Well
Cell lysate, µl (remaining in plate)	275	160	75	50	25
Protein lysis buffer, µl	92	53	25	16.7	8.3
Total, µl	**367**	**213**	**100**	**66.7**	**33.3**

3 RT-qPCR Analysis

Perform RT-qPCR according to the protocol included with the SingleShot Cell Lysis RT-qPCR Kit (see Table 6 for recommended kits) or in bulletin 6774.

For SingleShot Two-Step RT-qPCR Kits, use 4 µl of the RNA lysate per 20 µl cDNA synthesis reaction. Up to a maximum of 9 µl of lysate may be used for cDNA synthesis. No more than 10% of the qPCR reaction should consist of the cDNA synthesis reaction. For SingleShot One-Step RT-qPCR Kits, use up to 4 µl of RNA lysate per 20 µl reaction. For best results, use in conjunction with PrimePCR™ Gene Expression Assays (**bio-rad.com/primepcr**).

4 Western Blot Analysis

Denature each protein lysate for 1 min at 70–85°C and proceed with western blot analysis according to bulletin 6774. For gel electrophoresis, use a TGX Stain-Free Protein Gel (see Table 7 for recommended gels) and load 33 µl of protein lysate per lane. Alternatively, refer to bulletin 6390 for a detailed western blotting protocol using the V3 Western Workflow™. If following these instructions, start at Section 1 — Electrophoresis with stain-free gels.

5 Recommended RT-qPCR Kits and Protein Gels

Table 6. Recommended SingleShot Cell Lysis RT-qPCR Kits

Catalog #	Description
1725095	SingleShot™ SYBR® Green One-Step Kit
1725070	SingleShot Probes One-Step Kit
1725085	SingleShot™ SYBR® Green Kit
1725090	SingleShot Probes Kit

Table 7. Recommended TGX Stain-Free™ Protein Gels

Catalog #	Description
4568084	4–15% Mini-PROTEAN® TGX Stain-Free Protein Gels, 10 well, 50 µl
4568094	4–20% Mini-PROTEAN TGX Stain-Free Protein Gels, 10 well, 50 µl
4568124	Any kD™ Mini-PROTEAN TGX Stain-Free Protein Gels, 10 well, 50 µl
5678083	4–15% Criterion™ TGX Stain-Free Protein Gel, 12+2 well, 45 µl
5678093	4–20% Criterion TGX Stain-Free Protein Gel, 12+2 well, 45 µl
5678123	Any kD Criterion TGX Stain-Free Protein Gel, 12+2 well, 45 µl

See bulletin 6427 for additional TGX Stain-Free Protein Gels and other products used in the V3 Western Workflow.

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