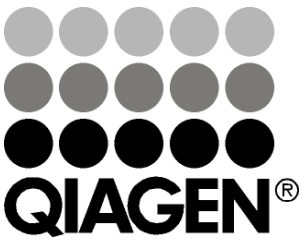
Second Edition October 2012

Qproteome® Cell Compartment Handbook

For the subcellular fractionation of proteomic samples



Sample & Assay Technologies

QIAGEN Sample and Assay Technologies

QIAGEN is the leading provider of innovative sample and assay technologies, enabling the isolation and detection of contents of any biological sample. Our advanced, high-quality products and services ensure success from sample to result.

QIAGEN sets standards in:

- Purification of DNA, RNA, and proteins
- Nucleic acid and protein assays
- microRNA research and RNAi
- Automation of sample and assay technologies

Our mission is to enable you to achieve outstanding success and breakthroughs. For more information, visit www.qiagen.com.

Contents

Kit Contents

Qproteome Cell Compartment Kit	
Catalog no.	**37502**
Number of preps	**Up to 10**
Lysis Buffer	10 ml
Extraction Buffer CE2	10 ml
Extraction Buffer CE3	5 ml
Extraction Buffer CE4	5 ml
Benzonase® Nuclease	80 µl
Protease Inhibitor Solution (100x)	300 µl
QIAshredder	10
Quick-Start Protocol	1

Storage

Benzonase® Nuclease, Lysis Buffer, and Extraction Buffers CE2 and CE3 should be stored at –20°C.

Protease Inhibitor Solution (100x) should be stored at 2–8°C.

Extraction Buffer CE4 should be stored at room temperature (15–25°C).

Intended Use

The Qproteome Cell Compartment Kit is intended for molecular biology applications. This product is not intended for the diagnosis, prevention, or treatment of a disease.

All due care and attention should be exercised in the handling of the products. We recommend all users of QIAGEN® products to adhere to the NIH guidelines that have been developed for recombinant DNA experiments, or to other applicable guidelines.

Safety Information

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate safety data sheets (SDSs). These are available online in convenient and compact PDF format at www.qiagen.com/safety where you can find, view, and print the SDS for each QIAGEN kit and kit component.

24-hour emergency information

Emergency medical information in English, French, and German can be obtained 24 hours a day from:

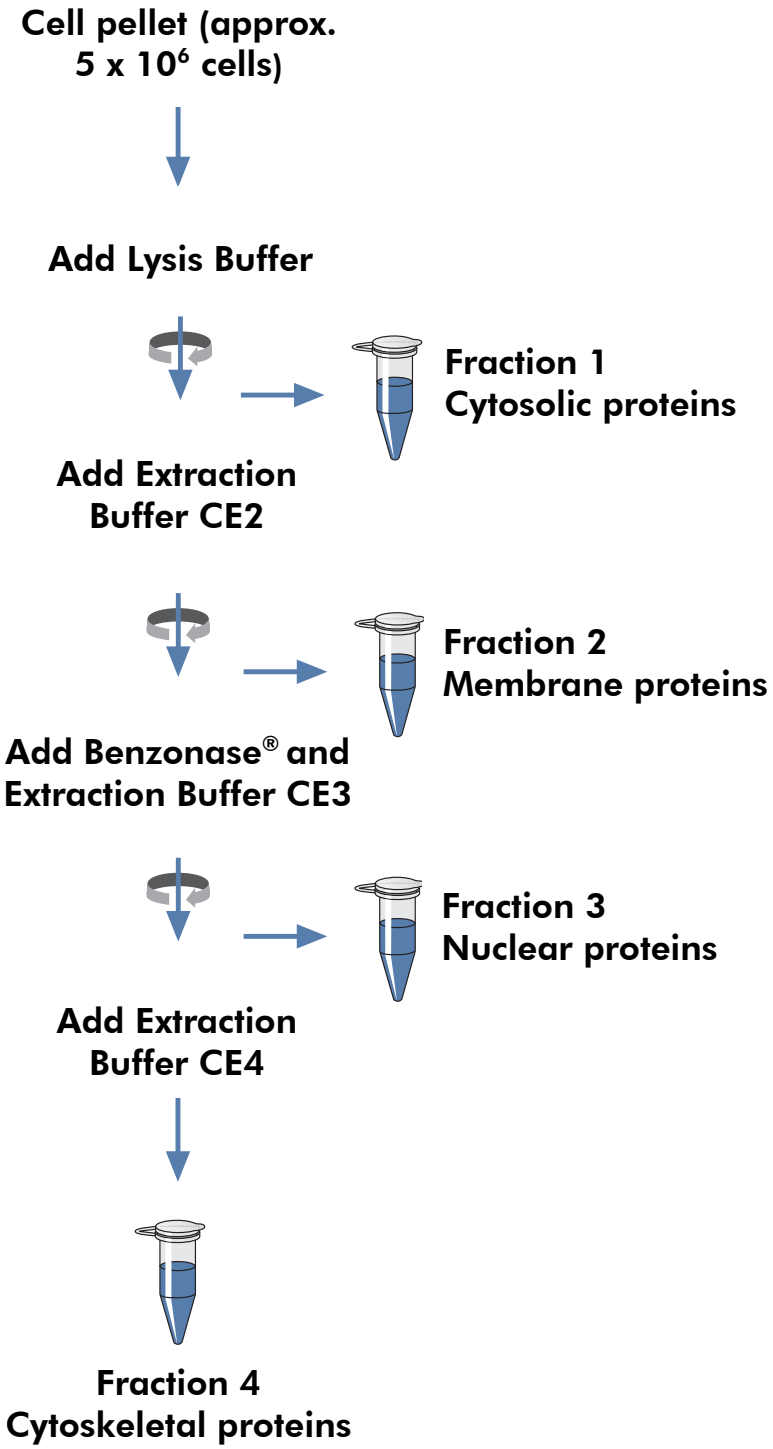
Poison Information Center Mainz, Germany

Tel: +49-6131-19240

Quality Control

In accordance with QIAGEN's ISO-certified Quality Management System, each lot of the Qproteome Cell Compartment Kit is tested against predetermined specifications to ensure consistent product quality.

Cell Compartment Kit Fractionation Procedure



Introduction

Eukaryotic cells are complex, well-ordered, and highly structured systems. The Qproteome Cell Compartment Kit is designed for fast and easy subcellular fractionation of intact eukaryotic cells and tissue. By sequential addition of different extraction buffers to a cell pellet, proteins in the different cellular compartments can be selectively isolated (see Table 1 and Figure 1, page 9).

Principle and procedure

Lysis Buffer is added to cells and selectively disrupts the plasma membrane without solubilizing it, resulting in the isolation of cytosolic proteins. Plasma membranes and compartmentalized organelles, such as nuclei, mitochondria, and the endoplasmic reticulum (ER), remain intact and are pelleted by centrifugation.

The pellet from the first step is resuspended in Extraction Buffer CE2, which solubilizes the plasma membrane, as well as all organelle membranes except the nuclear membrane. After solubilization, the sample is centrifuged. The supernatant contains membrane proteins and proteins from the lumen of organelles (e.g., the ER and mitochondria). The pellet consists of nuclei.

In the next step nuclei are solubilized using Extraction Buffer CE3 in which all soluble and most membrane-bound nuclear proteins are extracted. Addition of Benzonase® Nuclease allows the release of proteins tightly bound to nucleic acids (e.g., histones).

After another centrifugation, Extraction Buffer CE4 is used to solubilize all residual — mainly cytoskeletal — proteins in the pellet.

Fractions 1 to 3 contain proteins in their native state (Figure 1, page 9). Extraction Buffer CE4 is strongly denaturing and not compatible with isoelectric focusing. Proteins in all fractions must be desalted (e.g., by acetone precipitation, see page 16) before further analysis using isoelectric focusing (Figure 2, page 9).

Table 1. Subcellular protein fractionation from cellular components

Buffer	Used to isolate proteins from
Lysis Buffer	Cytosol
Extraction Buffer CE2	Membranes
Extraction Buffer CE3	Nucleus
Extraction Buffer CE4	Cytoskeleton

Starting material for one fractionation procedure is 5 x 10^6 cells or 20 mg tissue. The procedure has been used successfully for several different mammalian cell lines including HeLa, Jurkat, NIH-3T3, HEK293, and Cos. The tissue protocol has been tested with several rat tissues, including liver, heart, lung and brain. Table 2 and Table 3 give an overview of expected protein yields using different cell lines.

Subcellular fractionation of proteins enables:

- Enrichment of low-abundance species
- Definition of the subcellular localization of enzymes, regulatory, and structural proteins
- Monitoring of compartmental redistribution of biomolecules under basal and stimulated conditions

Table 2. Typical protein yields from cultured cells in cytosolic, membrane, nuclear, and cytoskeletal fractions*

Cell line	Cytosol	Membranes	Nucleus	Cytoskeleton
HeLa	490 µg	290 µg	160 µg	4 µg
Jurkat	380 µg	120 µg	110 µg	18 µg
NIH-3T3	430 µg	270 µg	150 µg	6 µg
Cos	450 µg	210 µg	130 µg	40 µg

* The yields in this table are mean values from 4 independent preparations of 5 x 10^6 cells. The % CV ranged between 5 and 26% for the first 3 fractions.

Table 3. Typical protein yields from different rat tissues in cytosolic, membrane, nuclear, and cytoskeletal fractions*

Tissue type	Cytosol	Membranes	Nucleus	Cytoskeleton
Brain	300 µg	194 µg	29 µg	0 µg
Liver	630 µg	360 µg	15 µg	0 µg
Heart	324 µg	122 µg	29 µg	28 µg
Lung	419 µg	42 µg	35 µg	0 µg

* Yields are mean values from 3 independent preparations of 20 mg tissue.

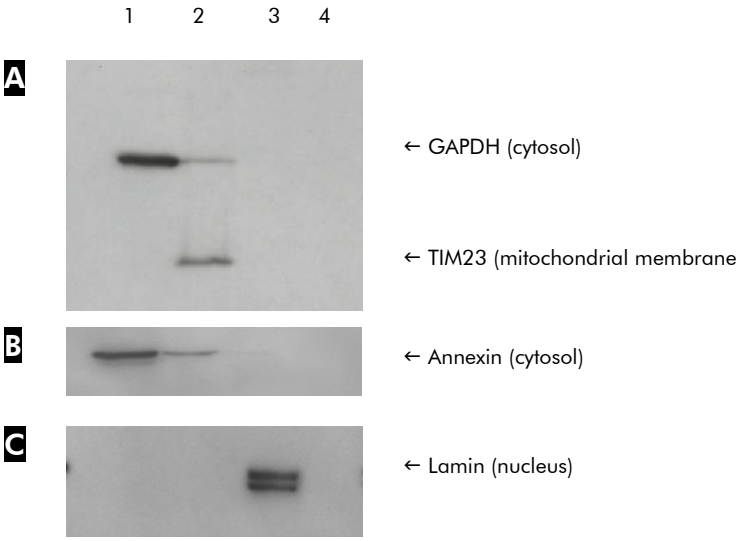


Figure 1. Subcellular fractionation of NIH-3T3 cells. Western blots of fractionated NIH-3T3 cells. Protein from fractions 1–4 (20 µg) was separated by SDS-PAGE. After western blotting, proteins specific to each fraction were detected using **A** GAPDH and TIM23, **B** annexin, and **C** lamin antibodies, and an HRP-conjugated secondary antibody with chemiluminescent detection.

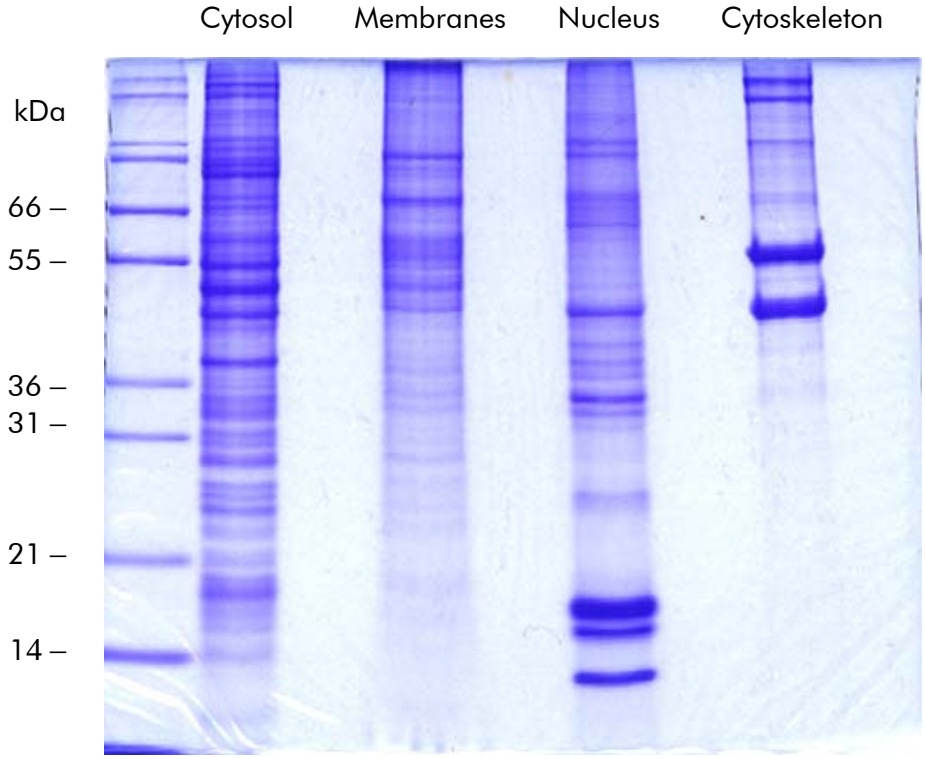


Figure 2. Separation of subcellular fractions. Coomassie-stained gel showing fractionation of NIH-3T3 cells using the Qproteome Cell Compartment Kit.

Equipment and Reagents to Be Supplied by User

All protocols

- Ice-cold PBS (50 mM NaH_2PO_4 , 150 mM NaCl, pH 7.2)
- Distilled water
- End-over-end shaker
- **Optional**: acetone stored at –20°C

Tissue protocol

- Disruption/homogenization device, e.g., TissueRuptor®
- **Optional**: acetone stored at –20°C

Protocol: Subcellular Fractionation of Cultured Cell Samples

This protocol is suitable for processing of 5 x 10^6 cells. **Note**: The extraction buffers contain components that may interfere with protein quantification assays. A precipitation step (e.g., using acetone, see page 16) to remove interfering substances is required to accurately determine protein concentrations.

Procedure

1. **Thaw Protease Inhibitor Solution (100x), Lysis Buffer, and Extraction Buffers CE2 and CE3. After thawing, mix well by vortexing and place on ice. For each fractionation procedure, prepare the volume of buffer supplemented with Protease Inhibitor Solution (100x) given in Table 4.**

Table 4. Volume of buffer required for each fractionation procedure

	Lysis Buffer	Buffer CE2	Buffer CE3	Buffer CE4
Required volume	1 ml	1 ml	0.5 ml	0.5 ml
Protease Inhibitor Solution (100x)	10 µl	10 µl	5 µl	–

2. **Transfer a cell suspension containing 5 x 10^6 cells into a 15 ml conical tube and centrifuge at 500 x *g* for 10 min at 4°C. Remove the supernatant carefully and discard it.**
3. **Resuspend the cell pellet in 2 ml ice-cold PBS by pipetting up and down with a 1 ml pipet tip and transfer the cell suspension into a microcentrifuge tube. Pellet cells by centrifuging at 500 x *g* for 10 min at 4°C. Remove the supernatant carefully and discard it.**
4. **Repeat step 3.**
5. **Resuspend the cell pellet in 1 ml ice-cold Lysis Buffer by pipetting up and down using a 1 ml pipet tip. Incubate for 10 min at 4°C on an end-over-end shaker.**

   Ensure that Protease Inhibitor Solution (100x) has been added to Lysis Buffer.
6. **Centrifuge the lysate at 1000 x *g* for 10 min at 4°C.**

7. **Carefully transfer the supernatant (fraction 1) into a fresh microcentrifuge tube. Store on ice.**

   This fraction primarily contains cytosolic proteins.

8. **Resuspend the pellet in 1ml ice-cold Extraction Buffer CE2 by pipetting up and down using a 1ml pipet tip. Incubate for 30 min at 4°C on an end-over-end shaker.**

   Ensure that Protease Inhibitor Solution (100x) has been added to Extraction Buffer CE2.

9. **Centrifuge the suspension at 6000 x *g* for 10 min at 4°C.**

10. **Carefully transfer the supernatant (fraction 2) into a fresh microcentrifuge tube. Store on ice.**

    This fraction primarily contains membrane proteins.

11. **Add 7 µl Benzonase® Nuclease and 13 µl distilled water to the pellet. Resuspend the pellet by gently flicking the bottom of the tube. Incubate for 15 min at room temperature (15–25°C).**

12. **Pipet 500 µl ice-cold Extraction Buffer CE3 into the tube and pipet up and down using a 1ml pipet tip. Incubate for 10 min at 4°C on an end-over-end shaker.**

    Ensure that Protease Inhibitor Solution (100x) has been added to Extraction Buffer CE3.

13. **Pellet insoluble material by centrifuging at 6800 x *g* for 10 min at 4°C.**

14. **Transfer the supernatant (fraction 3) into a fresh sample tube. Store on ice.**

    This fraction primarily contains nuclear proteins.

15. **Resuspend the pellet from step 13 in 500 µl Extraction Buffer CE4. Label the suspension fraction 4.**

    This fraction primarily contains cytoskeletal proteins.

Protocol: Subcellular Fractionation of Tissue Samples

This protocol is suitable for processing of 20 mg tissue.

Note: The extraction buffers contain components that may interfere with protein quantification assays. A precipitation step (e.g., using acetone, see page 16) to remove interfering substances is required to accurately determine protein concentrations.

For most tissues tested by QIAGEN (heart, lung, and liver) there is sufficient Lysis Buffer in the kit for a total of 10 preparations. However, some tissues (e.g., brain) require double the amount of Lysis Buffer and therefore a total of 5 preps can be performed per kit.

Procedure

1. **Thaw Protease Inhibitor Solution (100x), Lysis Buffer, and Extraction Buffers CE2 and CE3. After thawing, mix well by vortexing and place on ice. For each fractionation procedure, prepare the volume of buffer supplemented with Protease Inhibitor Solution (100x) given in Table 5.**

   When processing brain tissue sample prepare 2 ml Lysis Buffer.

Table 5. Volume of buffer required for each fractionation procedure

	Lysis Buffer	Lysis Buffer*	Buffer CE2	Buffer CE3	Buffer CE4
Required volume	1 ml	2 ml	1 ml	0.5 ml	0.5 ml
Protease Inhibitor Solution (100x)	10 µl	20 µl	10 µl	5 µl	–

* When processing brain tissue sample prepare 2 ml Lysis Buffer.

2. **Using a clean scalpel, cut the tissue into 3-4 pieces and wash with 1 ml ice-cold PBS buffer.**
3. **Place the tissue pieces into a clean 2 ml microcentrifuge tube and add 500 µl Lysis Buffer supplemented with Protease Inhibitor Solution (prepared in step 1).**
4. **Using the TissueRuptor, disrupt the tissue for 5 s at the lowest speed setting.**

   Do not disrupt the tissue for longer than 5 s. If clumps of tissue remain they will be filtered out in the next step.
5. **Transfer the tissue suspension to a QIAshredder homogenizer and centrifuge at 510 x *g* for 2 min at 4°C.**

   Ensure that Protease Inhibitor Solution (100x) has been added to Lysis Buffer.
6. **Resuspend the pellet by gently pipetting up and down and transfer the suspension to a clean 2 ml tube.**
7. **Add 500 µl Lysis Buffer (prepared in step 1).**

   IMPORTANT: When processing brain tissue add 1.5 ml Lysis Buffer in this step.
8. **Incubate the suspension on an end-over-end shaker for 10 min at 4°C.**
9. **Centrifuge the lysate at 4000 x *g* for 10 min at 4°C.**
10. **Carefully transfer the supernatant (fraction 1) into a fresh microcentrifuge tube. Store on ice.**

    This fraction primarily contains cytosolic proteins.
11. **Resuspend the pellet from step 10 in 1 ml ice-cold Extraction Buffer CE2 by pipetting up and down using a 1 ml pipet tip. Incubate for 30 min at 4°C on an end-over-end shaker.**

    Ensure that Protease Inhibitor Solution (100x) has been added to Extraction Buffer CE2.
12. **Centrifuge the suspension at 6000 x *g* for 10 min at 4°C.**
13. **Carefully transfer the supernatant (fraction 2) into a fresh microcentrifuge tube. Store on ice.**

    This fraction primarily contains membrane proteins.
14. **Add 7 µl Benzonase® Nuclease and 13 µl distilled water to the pellet. Resuspend the pellet by gently flicking the bottom of the tube. Incubate for 15 min at room temperature (15–25°C).**

15. **Pipet 500 µl ice-cold Extraction Buffer CE3 into the tube and pipet up and down using a 1ml pipet tip. Incubate for 10 min at 4°C on an end-over-end shaker.**

    Ensure that Protease Inhibitor Solution (100x) has been added to Extraction Buffer CE3.

16. **Pellet insoluble material by centrifuging at 6800 x *g* for 10 min at 4°C.**

17. **Transfer the supernatant (fraction 3) into a fresh sample tube. Store on ice.**

    This fraction primarily contains nuclear proteins.

18. **Resuspend the pellet from step 13 in 500 µl Extraction Buffer CE4. Label the suspension fraction 4.**

    This fraction primarily contains cytoskeletal proteins.

Protocol: Acetone Precipitation of Protein Fractions

This protocol is suitable for concentrating and desalting protein samples for downstream applications.

Procedure

1. **Add four volumes of ice-cold acetone to the protein fraction and incubate for 15 min on ice.**
2. **Centrifuge for 10 min at 12,000 x *g* in a pre-cooled microcentrifuge at 4°C. Discard the supernatant and air dry the pellet.**

   Do not overdry the pellet as this may make it difficult to resuspend.
3. **Depending on the application, resuspend the pellet in the required sample buffer.**

   For 2D-PAGE, an extra desalting step may be required.
4. **Resuspend the pellet from step 2 in 100 µl 8M urea.**
5. **Desalt the sample using a gel filtration device (e.g., Bio-Spin® 6, Bio-Rad cat. no. 732-6227).**
6. **Repeat steps 1 to 3.**

Troubleshooting Guide

This troubleshooting guide may be helpful in solving any problems that may arise. For more information, see also the Frequently Asked Questions page at our Technical Support Center: www.qiagen.com/FAQ/FAQList.aspx. The scientists in QIAGEN Technical Services are always happy to answer any questions you may have about either the information and protocols in this handbook or sample and assay technologies (for contact information, see back cover or visit www.qiagen.com).

	Comments and Suggestions
Inconsistent results in protein quantification assays	The extraction buffers contain components that may interfere with protein quantification assays. A precipitation step (e.g., using acetone, see page 16) to remove interfering substances is required to accurately determine protein concentrations.
Marker proteins do not appear in expected fraction/appear in different fraction	The amount of cells or tissue processed was too high. The protocol is suitable for processing of 5 x 10^6 cells or 20 mg tissue.
	Marker protein may shuffle between compartments, for example, upon apoptotic stimulus cytochrome c can be transported from mitochondria (fraction 2) to the cytosol (fraction 1).
A precipitate forms when storing Extraction Buffer CE4 on ice.	The precipitate can be redissolved by heating the buffer to 37°C with agitation and cooling to room temperature (15–25°C).
Protease Inhibitor does not thaw at room temperature	Heat the protease inhibitor solution to 37°C with agitation and cool to room temperature.

Ordering Information

Product	Contents	Cat. no.
Qproteome Cell Compartment Kit	For up to 10 subcellular fractionations: Extraction buffers, Protease Inhibitor Solution, Benzonase® Nuclease	37502
Related products		
TissueRuptor	Handheld rotor–stator homogenizer, 5 TissueRuptor Disposable Probes	9001271* 9001272† 9001273‡ 9001274§
TissueRuptor Disposable Probes (25)	25 nonsterile plastic disposable probes for use with the TissueRuptor	990890
QIAshredder (50)	50 disposable cell-lysate homogenizers for use in nucleic acid minipreps, caps	79654

* 120 V, 60 Hz (for North America and Japan); † 235 V, 50/60 Hz (for Europe, excluding UK and Ireland); ‡ 235 V, 50/60 Hz (for UK and Ireland); § 235 V, 50/60 Hz (for Australia)

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual. QIAGEN kit handbooks and user manuals are available at www.qiagen.com or can be requested from QIAGEN Technical Services or your local distributor.

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1073571 10/2012

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