

# GPCR Extraction & Stabilization Reagent

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 **WARNING!** Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from [thermofisher.com/support](https://www.thermofisher.com/support).

## Product description

The Thermo Scientific™ GPCR Extraction & Stabilization Reagent is used for the extraction and stabilization of G-coupled Protein Receptors (GPCRs) and other membrane-associated proteins from cultured mammalian cells or tissue. The extraction reagent encapsulates the receptor in a detergent micelle optimized specifically to ensure receptor function for up to 1 week when stored at 4°C and 1 month when stored at -20°C post extraction.

There are many challenges to stabilizing receptors in their native and functional form, post extraction, making studies outside of the cell membrane extremely difficult. This extraction reagent enables efficient extraction of functional receptors from mammalian cells or tissue in 1-2 hours. Most functional GPCR assays have to be performed immediately after extraction of the receptor, with activity rapidly degrading over time. The GPCR Extraction & Stabilization Reagent increases receptor stability giving researchers more flexibility with their samples.

## Contents

Product	Contents	Storage
GPCR Extraction & Stabilization Reagent	100 mL Contains sufficient extraction and stabilization reagent for approximately 100 mammalian cell pellet fractions containing $1 \times 10^7$ cells each or 100 tissue samples containing 50-100 mg of tissue.	Store at 4°C.

## Important product information

- For optimal results, include protease and phosphatase inhibitors (e.g., Product No. 78440) in the Extraction Reagent.
- To directly quantify proteins, use a protein assay such as the Thermo Scientific™ Pierce™ BCA Protein Assay Kit (Product No. 23225), Micro BCA™ Protein Assay Kit (Product No. 23235), Pierce™ 660nm Protein Assay Kit (Product No. 22662), or Pierce™ Rapid Gold BCA Protein Assay Kit (Product No. A53226).
- Samples are compatible with SDS-PAGE. Use the Thermo Scientific™ Pierce™ SDS-PAGE Sample Prep Kit (Product No. 89888) if the protein of interest is in low abundance and a large volume is required for adequate detection.

## Materials required but not provided

- Protease and phosphatase inhibitors (e.g., Product No. 78440)
- Temperature-controlled mixer
- Cell scraper to remove adherent cells from plates
- 1.5 mL microcentrifuge tubes
- For tissues and over-expression systems, a 2 mL Dounce Tissue Grinder (e.g., Kontes or Wheaton Tenbroeck) is required.

## Procedure for membrane protein extraction from different sample types

### Adherent mammalian cells

- Wash cells twice in 10-15 mL of cold PBS on the plate.
- Add 1 mL of cold PBS and scrape cells off the surface of the plate with a cell scraper.
- Resuspend  $1 \times 10^7$  cells in 1 mL of cold PBS. Centrifuge harvested cell suspension at  $500 \times g$  for 5 minutes at 4°C.
- Carefully remove and discard the supernatant. Resuspend the cells in 1 mL of cold PBS and transfer to a 1.5 mL centrifuge tube. Centrifuge at  $500 \times g$  for 5 minutes at 4°C and discard supernatant.
- Add 1 mL of cold GPCR Extraction & Stabilization Reagent to the cell pellet. Pipet up and down 10-15x and then vortex briefly to obtain a homogeneous cell suspension. Incubate 30 minutes at 4°C with constant mixing.

**Note:** For over-expression systems, mild mechanical disruption is required at this step for maximum results (i.e. for Expi293 systems homogenization using a Dounce homogenizer or handheld tissue grinder increases yield of active receptor).

- Centrifuge tubes for 20 minutes at  $16,000 \times g$  at 4°C. Carefully remove the supernatant containing stabilized protein receptors and transfer to a new tube.
- Immediately proceed to downstream application or store extract aliquots for future use at 4°C for up to 1 week or -20°C for up to 1 month with minimal loss in receptor function.

## Suspension mammalian cells

1. Harvest  $1 \times 10^7$  cells by centrifugation at  $500 \times g$  for 5 minutes.
2. Wash harvested cell pellet twice with 10-15 mL of cold PBS and centrifuge at  $500 \times g$  for 5 minutes at  $4^\circ\text{C}$ .
3. Carefully remove and discard the supernatant. Resuspend the cells in 1 mL of cold PBS and transfer to a 1.5 mL centrifuge tube. Centrifuge at  $500 \times g$  at  $4^\circ\text{C}$  for 5 minutes and discard supernatant.
4. Add 1 mL of cold GPCR Extraction & Stabilization Reagent to the cell pellet. Pipet up and down 10-15x and then vortex briefly to obtain a homogeneous cell suspension. Incubate 30 minutes at  $4^\circ\text{C}$  with constant mixing.

**Note:** For over-expression systems, mild mechanical disruption is required at this step for maximum results (i.e. for Expi293 systems homogenization using a Dounce homogenizer or handheld tissue grinder increases yield of active receptor).

5. Centrifuge tubes for 20 minutes at  $16,000 \times g$  at  $4^\circ\text{C}$ . Carefully remove the supernatant containing stabilized protein receptors and transfer to a new tube.
6. Immediately proceed to downstream application or store extract aliquots for future use at  $4^\circ\text{C}$  for up to 1 week or  $-20^\circ\text{C}$  for up to 1 month with minimal loss in receptor function.

## Tissue

1. Place 50-100 mg of tissue in a 5 mL tube. Add 4 mL of cold PBS to the tissue, vortex briefly, and discard wash.
2. Transfer to a 2 mL Dounce homogenizer and cut the tissue into small pieces with a pair of scissors. Add 1 mL of cold GPCR Extraction & Stabilization Reagent to the tissue and homogenize until an even suspension is obtained (10-15 strokes).

**Note:** Homogenization using a hand-help tissue grinder (i.e., polytron) is optional, but may cause foaming.

3. Transfer homogenate to a new tube, incubating for 30 minutes at  $4^\circ\text{C}$  with constant mixing.

*(Optional)* Remove non-homogenized tissue using the protocol provided with the Thermo Scientific™ Pierce™ Tissue Strainers (Product No. 87791) before pelleting cell debris.

4. Centrifuge at  $16,000 \times g$  for 20 minutes at  $4^\circ\text{C}$  to pellet cell debris. Carefully remove the supernatant containing stabilized protein receptors and transfer to a new tube.
5. Immediately proceed to downstream application or store extract aliquots for future use at  $4^\circ\text{C}$  for up to 1 week or  $-20^\circ\text{C}$  for up to 1 month with minimal loss in receptor function.

## Troubleshooting

Observation	Possible cause	Recommended action
Cells not pelleting at the bottom of the microcentrifuge tube post-PBS wash.	Spin speed was too low.	Increase spin speed to $500-1000 \times g$ .
	Spin time was too short.	Increase 5-minute spin time to 10 minutes.
Lysate concentration is too low.	Cell viability was below 70%.	Ensure that cells have viability greater than 70%.
	Cells were not confluent enough.	Ensure cells are 80-90% confluent.
	Cell count was too low.	Ensure you are extracting $1 \times 10^7$ cells per mL of extraction buffer. If cell count is lower, decrease the amount of buffer used for extraction (i.e., use 0.5 mL of extraction buffer for $5 \times 10^6$ cells).
Lysate is viscous.	Volume of extraction buffer was too low.	Increase volume of extraction buffer (i.e., use 2 mL of extraction buffer for $2 \times 10^7$ cells).
Low yield of active receptor.	Poor solubilization from cell membrane.	Use mechanical disruption (i.e. Dounce homogenization) prior to incubation in extraction reagent.
	Mechanical disruption was too aggressive.	Do not use sonication for mechanical disruption. Disrupt with Dounce homogenizer or electric tissue grinder instead.

## Related products

Product	Product No.
Halt™ Protease Inhibitor Cocktail, EDTA-Free (100X)	87785
Halt™ Protease Inhibitor Cocktail (100X)	87786
Halt™ Protease and Phosphatase Inhibitor Cocktail, EDTA-Free (100X)	78441
Halt™ Protease and Phosphatase Inhibitor Cocktail (100X)	78440
Halt™ Phosphatase Inhibitor Cocktail (100X)	78420
Pierce™ Protease Inhibitor Tablets	88665-9
BCA Protein Assay Kit	23225
Pierce™ SDS-PAGE Sample Prep Kit	89888
Pierce™ Detergent Removal Spin Columns, 0.5 mL	87777
Subcellular Protein Fractionation Kit for Cultured Cells	78840
Subcellular Protein Fractionation Kit for Tissue	87790
Pierce™ Tissue Strainers, 250 $\mu\text{m}$ , 2.5 mL, 50 units	87791

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