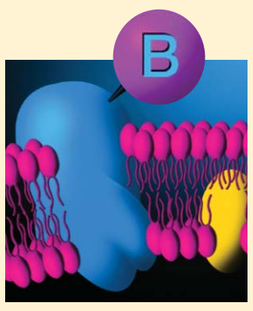
Thermo Scientific Tools for

Cellular Proteomics



The Cell Surface

Normal cell function, and its contribution to overall physiology, depends on the proper response of cells to stimuli or extracellular signals. All such stimuli are received, processed and delivered by receptors, gates and channels that are embedded in or bound to the cell membrane. Receptor proteins in the cell membrane receive signals from the environment or from other cells and transmit them internally to effect a response, a process termed signal transduction. Small GTPases of the Ras superfamily are monomeric guanine nucleotide-binding proteins that serve as molecular switches to regulate growth, morphogenesis, cell mobility, axonal guidance, cytokinesis and trafficking.

Thermo Scientific Active GTPase Pull-Down and Detection Kits measure the activation of Rac1, Cdc42, Ras, Rho or Rap1 small GTPases by isolating them via their specific downstream effectors. The respective binding domain of the downstream effector for each small GTPase is expressed as a GST-fusion protein which, when immobilized on a resin, is used to pull down the active or GTP-bound GTPase. The kits also include a specific primary antibody for Western blot detection of the purified GTPase.

About 3,000 human genes are believed to encode for proteins that are displayed at the cell surface. Secreted and cell surface proteins are often more difficult to isolate and identify than proteins that are localized in intracellular compartments. The Thermo Scientific Cell Surface Protein Isolation Kit provides a convenient and efficient method for isolating cell surface proteins from cultured mammalian cells. Adherent or suspended cells are first labeled with Sulfo-NHS-SS-Biotin, a cleavable biotinylation reagent. The cells are subsequently lysed with a mild detergent, and labeled proteins are isolated with Thermo Scientific NeutrAvidin* Resin. Isolated proteins can be analyzed by Western blot, allowing for differential expression analysis between treated and non-treated cells or between two or more cell lines.

Ordering Information

Product #	Description	Product #	Description
89854	Active Rho Pull-Down and Detection Kit	89857	Active Cdc42 Pull-Down and Detection Kit
89855	Active Ras Pull-Down and Detection Kit	89872	Active Rap1 Pull-Down and Detection Kit
89856	Active Rac1 Pull-Down and Detection Kit	89881	Cell Surface Protein Isolation Kit

Visit thermo.com/pierce for kit components

Membrane Proteins

Membrane proteins comprise approximately 30% of the eukaryotic proteome, and elucidating and controlling their functions are important goals of drug discovery research. Membrane proteins act as transporters, channels, receptors and enzymes, structural and cell-adhesion anchoring domains, accumulators, and transducers of energy. However, membrane proteins are difficult to isolate because of their hydrophobicity, basic nature and large size. Thermo Scientific Pierce Research Products include a comprehensive line of cell lysis reagents and kits designed to maximize extraction of target proteins from cell membranes, organelles and cytoplasm. The Thermo Scientific Mem-PER* Eukaryotic Membrane Protein Isolation Kit effectively isolates single and multiple transmembrane proteins from eukaryotic cells or tissue.

Ordering Information

Product #	Description
89826	Mem-PER Eukaryotic Membrane Protein Isolation Kit

Visit thermo.com/pierce for kit components.

Cell Lysis and Protein Extraction

Most experimental and assay methods of proteomics analysis begin with some form of cell lysis to obtain an extract of soluble cellular protein contents. The quantity and quality of data that can be derived from analysis of extracted proteins directly depends on the amount and functional integrity of the proteins that result from the extraction process. Some extraction methods may be efficient at cell lysis and solubilization of cell contents but are protein-denaturing, thereby preventing detection and analysis of native protein interactions. Ease of analysis also depends upon compatibility of the lysis and extraction reagents with the downstream technique. For example, some extraction reagents contain ionic detergents and reducing agents that interfere with protein assays, affinity purification, 2-D electrophoresis and other methods.

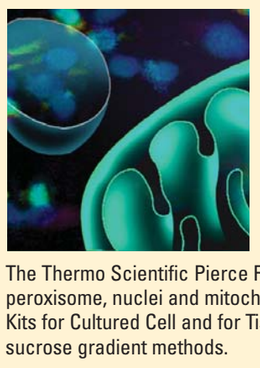
Thermo Scientific Pierce Cell Lysis Reagents provide for convenient, efficient and broadly compatible cell lysis and protein extraction of mammalian cells and tissues. Thermo Scientific M-PER* Mammalian Protein Extraction Reagent is ideal for use with suspension or adherent cultured cells of many types and mammalian species. M-PER Extracts are directly compatible with many assays including co-immunoprecipitation (co-IP), chemical labeling, 2-D electrophoresis and other methods. Thermo Scientific T-PER* Tissue Protein Extraction Reagent provides for efficient and mild protein extraction from tissues using homogenization.

Protease and phosphatase inhibitors are also essential for recovering useable protein samples from cell or tissue lysis. These reagents prevent enzymes that are released from subcellular vesicles from degrading polypeptide sequences or altering phosphorylation states of proteins in an extract. Thermo Scientific Halt* Protease and Phosphatase Inhibitor Cocktails provide superior protection and ease-of-use compared to conventional tablet reagents.

Ordering Information

Product #	Description	Product #	Description
78501	M-PER Mammalian Protein Extraction Reagent	78442	Halt Protease and Phosphatase Single-Use Inhibitor Cocktail
78510	T-PER Tissue Protein Extraction Reagent	78430	Halt Protease Inhibitor Single-Use Cocktail (100X)
89901	RIPA Buffer	78420	Halt Phosphatase Inhibitor Cocktail

Visit thermo.com/pierce for kit components.



Organelle Isolation

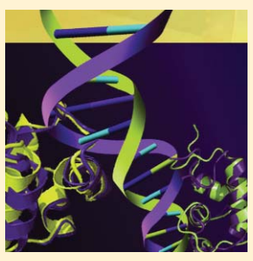
Mitochondria are found in most eukaryotic cells. In addition to energy metabolism, mitochondria are involved in cell signaling, cell differentiation, cell death and control of the cell cycle and cell growth. Defects in mitochondria have been associated with apoptosis, aging and diseases such as Parkinson's disease, diabetes and Alzheimer's disease. Of the 1,500 human mitochondrial proteins involved in mitochondrial function, most are nuclear-coded, synthesized in the cytosol and targeted to the mitochondria. This "mitoproteome" can be difficult to study if the method to isolate intact mitochondria is inconsistent or does not allow multiple samples to be processed concurrently.

The Thermo Scientific Pierce Research Product Line includes several kits optimized for the isolation of intact organelles. Kits for lysosome, peroxisome, nuclei and mitochondria enable researchers to study the specific organelle of interest. The patented Mitochondria Isolation Kits for Cultured Cell and for Tissue† gently and effectively isolate intact mitochondria without the need for Dounce homogenization or sucrose gradient methods.

Ordering Information

Product #	Description	Product #	Description
89839	Lysosome Enrichment Kit for Tissues and Cultured Cells	89874	Mitochondria Isolation Kit for Cultured Cells
89840	Peroxisome Enrichment Kit for Tissue	89801	Mitochondria Isolation Kit for Tissue
89841	Nuclei Enrichment Kit for Tissue		

Visit thermo.com/pierce for kit components.



Nuclear Targets

Although the "central dogma" describes the top-down flow of information from nuclear DNA to transcription of RNA to translation of proteins, the entire process is intimately controlled by bottom-up regulation from the cellular proteomic environment. For example, transcription factors are proteins activated by cell surface receptor-ligand interactions and other intercellular signals and involved in the transcription of genes into RNA.

Measurement and analysis of transcription factors and other protein:DNA binding interactions of the nuclear proteome are facilitated by isolation or enrichment of the nuclear protein component of a sample. The Thermo Scientific NE-PER* Nuclear and Cytoplasmic Extraction Kit provides for efficient cell lysis and isolation of soluble cytoplasmic and nuclear proteins into two separate fractions. Specific transcription factors in the nuclear extract can be accurately measured in a microplate format using a Chemiluminescent Transcription Factor Assay Kit for NFκB p50, NFκB p65 or c-Fos.

The Thermo Scientific LightShift* Chemiluminescent EMSA Kit enables accurate and highly sensitive, non-isotopic detection of DNA binding proteins in nuclear protein extracts. Nearly any DNA-binding protein can be measured in this electrophoretic mobility shift assay (EMSA) by supplying the appropriate biotin end-labeled DNA binding sequence. Unlike traditional EMSAs, the LightShift Kit is formatted for chemiluminescent detection on a simple mini-blot.

Ordering Information

Product #	Description	Product #	Description
78833	NE-PER Nuclear and Cytoplasmic Extraction Kit	89860	c-Fos Chemiluminescent Transcription Factor Assay Kit
89858	NFκB p50 Chemiluminescent Transcription Factor Assay Kit	20148	LightShift Chemiluminescent EMSA Kit
89859	NFκB p65 Chemiluminescent Transcription Factor Assay Kit	89880	Chemiluminescent Nucleic Acid Detection Module

Visit thermo.com/pierce for kit components

Extracellular Targets

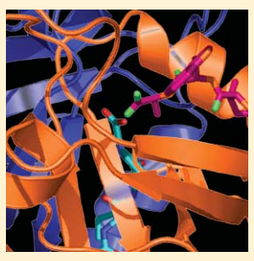
Cells synthesize, present, release and secrete a variety of cytokines, soluble receptors, hormones and byproducts to the extracellular environment. Synthesized in response to a variety of intracellular and extracellular signals, these products act locally (autocrine and paracrine effects) or at a distance (endocrine effects) to communicate and regulate cellular functions by interaction with cell surface receptors of specific cell signaling systems. Because their primary functions are mediated by secretion in extracellular fluids, cytokines and other such products are frequently assayed in serum, plasma and cell culture supernatants.

Thermo Scientific Pierce Protein Research Products include ELISA kits, matched antibody pairs and recombinant proteins for analysis of cytokines (interferons, interleukins, tumor necrosis factors, etc.), cytokine receptors (soluble and cell surface) and selected other extracellular targets (cyclic AMP, cyclic GMP, nitric oxide, leukotriene B4, prostaglandin E4, matrix metalloproteases, etc.). These products allow scientists to characterize disease states and drug responses by measuring production and extracellular expression of soluble proteins relevant to inflammation and other critical cellular functions.

Ordering Information

Product #	Description	Product #	Description
EHIFNG	Human IFN gamma ELISA Kit	EH2IL65	Human IL-6 ELISA Kit
EHIFNG2	Human IFN gamma ELISA Kit	EH3TNFA	Human TNF alpha ELISA Kit
EHIFNG5	Human IFN gamma ELISA Kit	EH3TNFA2	Human TNF alpha ELISA Kit
EH2IL6	Human IL-6 ELISA Kit	EH3TNFA5	Human TNF alpha ELISA Kit

Note: Selected mouse, rat, porcine and bovine kits and antibodies are also available.
Visit thermo.com/pierce for kit components.



Protein Interactions

Interactions among proteins are essential to most cellular functions and critical to a full understanding of cellular biology. For example, signals originating at the surface of a cell are transmitted through the cell membrane and to the appropriate intracellular target via protein interactions in the signal transduction cascade. Understanding these interactions is key to unlocking the mysteries of normal biological processes as well as disease progression, which can provide the basis for new therapeutic approaches.

Thermo Scientific Pierce Protein Research Products include a variety of tools to discover, confirm and characterize protein interactions. The photoreactive amino acid analogs L-Photo-Leucine and L-Photo-Methionine can be incorporated into proteins in a living cell and then used to crosslink interacting proteins in their native cellular context. Specific protein complexes can be isolated with Thermo Scientific Pierce Co-immunoprecipitation Kits to search for interacting proteins, confirm putative interactions or allow further analysis of the individual proteins that make up a particular complex. The analysis of co-IP results is enhanced by using the Thermo Scientific Clean-Blot IP Detection Kit, which easily binds to a primary antibody used for Western blot detection but does not cross-react with the antibody used for precipitation. Thermo Scientific Pierce Crosslinkers such as BS^3, DSS and formaldehyde are popular choices for fixing transient protein interactions in place for isolation and analysis. Other kits for pull-down assays, far-Western blots and label transfer are also available to study protein interactions.

Ordering Information

Product #	Description	Product #	Description
22610	L-Photo-Leucine (L-2-amino-4, 4'-azi-pentanoic acid)	23605	Pierce Mammalian Co-Immunoprecipitation Kit
22615	L-Photo-Methionine (L-2-amino-5, 5'-azi-hexanoic acid)	21580	BS^3 (Bis[sulfosuccinimidyl] suberate)
30030	Dulbecco's Modified Eagle's Limiting Medium (DMEM-LM)	21585	BS^3, No-Weigh format
23600	Pierce Co-Immunoprecipitation Kit	28908	16% Formaldehyde (w/v), Methanol-free

Visit thermo.com/pierce for kit components.

Post-Translational Modifications

Cells rely on post-translational modification (PTM), a physiochemical event, to control vital processes such as protein function, regulation and expression. Among the several hundred known types of PTMs, protein phosphorylation, glycosylation and ubiquitination are the most intensely studied. Characterization of these modifications, although challenging, provides invaluable insight into the cellular functions underlying etiological processes.

The first challenge in studying post-translationally modified proteins is to separate them from the sample source without damaging the structural integrity or functionality of modified proteins. Thermo Scientific Pierce Protein Research Products include a specialized line of kits and reagents that enable efficient extraction, purification, enrichment and detection of phosphorylated, ubiquitinyated and glycosylated proteins.

Ordering Information

Product #	Description	Product #	Description
90003	Pierce Phosphoprotein Enrichment Kit	24565	O-GlcNAc Western Blot Detection Kit
89853	Pierce Phosphopeptide Isolation Kit	53074	Krypton* Glycoprotein Staining Kit
89804	Glycoprotein Isolation Kit, ConA	24562	GelCode* Glycoprotein Staining Kit
89805	Glycoprotein Isolation Kit, WGA	24550	GelCode Phosphoprotein Staining Kit
89899	Ubiquitin Enrichment Kit		

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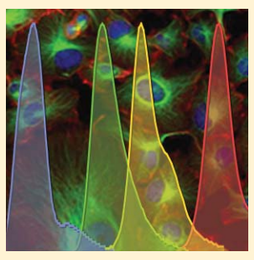


Cellular Imaging and Analysis

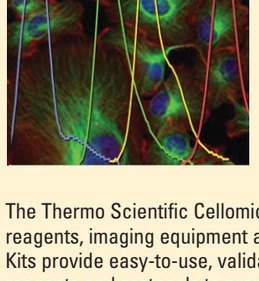
Tools for the analysis of cellular processes consist of several labeling technologies to facilitate analysis by fluorescence microscopy, flow cytometry, Western blotting, high-content screening and other array platforms. In recent years a growing appreciation for the complexity of cellular processes has increased demand for higher resolution probing and imaging technologies that are capable of measuring multiple parameters simultaneously and/or the distribution and abundance of specific cellular targets over time. We have met the demand for such a technology by developing Thermo Scientific DyLight* Fluors, a complete family of high-intensity, photostable fluorescent dyes for labeling antibodies and other molecular probes.

DyLight Fluors provide superior performance to traditional fluorophores such as fluorescein and enable multiplexing analysis in several non-overlapping excitation/emission channels. DyLight Fluor-conjugated secondary antibodies are the basis for Thermo Scientific Cellomics* High Content Screening Reagent Kits. Conjugated mouse and rabbit secondary antibodies are available, as well as both NHS-ester-activated and maleimide-activated forms, providing for efficient and specific labeling of primary amines and sulfhydryl groups, respectively.

Ordering Information

Product #	Description	Product #	Description
46407	DyLight 549 NHS Ester	24565	O-GlcNAc Western Blot Detection Kit
89853	Pierce Phosphopeptide Isolation Kit	53074	Krypton Glycoprotein Staining Kit
89804	Glycoprotein Isolation Kit, ConA	24562	GelCode Glycoprotein Staining Kit
89805	Glycoprotein Isolation Kit, WGA	24550	GelCode Phosphoprotein Staining Kit
89899	Ubiquitin Enrichment Kit		

Note: A similar range of reagents, kits and conjugates is available for all DyLight Fluors.



High-Content Screening in Drug Discovery

High-content screening (HCS) is emerging as an indispensable tool for analysis of cellular biological complexity in relation to drug discovery. HCS involves visualization of target protein expression and distribution in a cell population, single cell or subcellular structure and data analysis with image-analysis tools. The ability of HCS to measure multiple parameters related to spatial and temporal changes in populations of individual cells allows for measurement of experimental effects from a system perspective. HCS is widely used in all stages of target-based drug discovery that involve the study of cells, including target discovery, drug screening in cell-based assays, early safety evaluation, mode-of-action analysis and *in vivo* studies to monitor cell fate.

The Thermo Scientific Cellomics HCS Platform comprises a total solution for high-content screening. The platform includes fluorescent reagents, imaging equipment and software for image analysis, data management, automation and informatics. Cellomics HCS Reagent Kits provide easy-to-use, validated probes (fluorescent stains, target-specific primary antibodies and fluorescent secondary antibodies), reagents and protocols to prepare imaging-quality samples for HCS.

Available Thermo Scientific Cellomics HCS Reagent Kits.

Category of Analysis	Selected List of Kit Targets
Cytotoxicity and Apoptosis	Caspase 3, Caspase 9, Cleaved PARP, Cytochrome C, LC3B (autophagy), Poly-ubiquitin and other parameters
Genotoxicity, DNA Damage and Repair	Ku70/80, MDM2, Micronucleus, p21, p53, ATM, Chk2, H2AX
Inflammation and Cell Stress	CHOP/GADD153, COX-2, FKBP52, Heme Oxygenase 1, Hsp27, Hsp60, Hsp70, Hsp90, iNOS, MnSOD, NFAT-1, NFκB, oxidative stress, p38, 4E-BP1, c-Jun, S6, STAT1, 2 & 3
Cell Signaling and Transcription Factors	ATF-2, β–Catenin, ERK, FOXO1A & 3A, HIF-1α, AKT, CREB, GSK-3, JNK, PKA, PKCα, Smad3
Cell Cycle and Proliferation	BrdU, Cyclin B1, Ki67, Histone H3, PLK1, Rb, and other parameters
Cell Morphology and Phenotypic Changes	F-actin, Tubulin, Whole Cell Stains, and several other parameters
Accessory Reagents	Whole cell stains, fluorescent 2° antibodies, etc.

Kits are specific for active (e.g. phosphorylated) forms of target proteins.

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