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APPENDIX



Glossary

Common terms used in proteomics research

BRET (Bioluminescence Resonance Energy Transfer):

This technology is based on the transfer of resonant energy from a bioluminescent donor protein to a fluorescent acceptor protein using *Renilla* luciferase (*Rluc*) as the donor and a mutant of the Green Fluorescent Protein (GFP) as the acceptor molecule.

Cell-free expression: Cellular extract containing all the components required for the coupled transcription/translation of protein-coding DNA sequences.

Co-immunoprecipitation: An experiment designed to affinity purify a bait protein antigen together with its binding partner using a specific antibody against the bait.

Denature: To cause a protein to fold or unfold into a structure other than its native 3-D conformation.

Endoplasmic reticulum (ER): A membrane system that extends throughout the cytoplasm and is involved in the synthesis, processing, transport, and secretion of proteins.

EMSA: EMSA (Electrophoretic Mobility Shift Assay), also known as gel retardation/gel shift, the assay is based on the fact that protein:DNA complexes migrate more slowly through a native polyacrylamide or agarose gel than unbound DNA. The individual protein:DNA complexes can be visualized as discreet bands within the gel using chemiluminescence or radioisotopic detection.

Expression profiling: A high-throughput method for evaluating the degree and timing of gene expression in a cell or tissue.

FRET: FRET (Fluorescence Resonance Energy Transfer) is a technique that can measure interactions between two proteins in vivo. The occurrence of FRET can be observed by exciting the sample at the donor excitation wavelength while measuring fluorescence intensities emitted at the wavelengths corresponding to the emission peaks of the donor compared to those of the acceptor. Donor emission intensity decreases while acceptor intensity increases. When the donor and acceptor are in close proximity, FRET occurs and the acceptor emission is observed.

Functional proteomics: The large-scale study of protein function, especially protein:protein interaction networks, biochemical pathways, and post-translational modifications.

GST (glutathione-S-transferase): A 26kDa fusion tag developed from *Schistosoma japonicum* that has a strong affinity for glutathione covered matrices. GST-fusion protein binding to glutathione is reversible, allowing efficient elution of the bound GST-fusion protein by addition of reduced glutathione to the elution buffer.

Glycoprotein: A protein with covalently bound carbohydrates.

Glycosylation: Post-translational addition of carbohydrate groups to a molecule. Glycosylation of proteins occurs via the amide group within the sequence Asn-X-Ser/Thr (or through the hydroxyl of the serine or threonine residue in the sequence).

HaloTag Interchangeable Labeling

Technology: A novel tool for imaging live or fixed mammalian cells that express the HaloTag protein or protein fusions, for analyzing post-translational modification of labeled fusion proteins, and for isolating proteins and protein complexes. The technology is based on the efficient formation of a covalent bond between a specially designed ligand and the protein encoded by the HaloTag gene.

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Heat-shock protein: A protein synthesized in response to cellular stress, including high temperature. Heat-shock proteins function as molecular chaperones to protect proteins from mis-folding.

Kozak sequence: A DNA sequence that surrounds the ATG start signal for the translation of an mRNA.

Mass spectrometer (MS): An instrument that determines the exact mass of charged particles or ions by measuring the flight path through a set of magnetic and electric fields. Mass spectrometers specialized for protein and peptide sequencing are used for high-throughput identification.

Nuclear magnetic resonance (NMR): A spectroscopic technique used to determine the 3-D structure of small- to medium-sized proteins. NMR is based on resonant absorption of electromagnetic radiation by the magnetic dipole moments of atomic nuclei in an applied magnetic field.

Open Reading Frame (ORF): The DNA sequence between the translation start signal and the termination codon that can be translated into a protein.

Peptide: Two or more amino acids joined by a peptide bond.

Peptide bond: An amide bond formed between two amino acids by the linkage of the amino group of one amino acid to the carboxyl group of a second amino acid.

Peptide map, peptide fingerprint: A pattern produced by hydrolysis of a protein and 2-D mapping of the resulting peptide fragments.

Phage display (peptide phage display): A technique that fuses peptides to capsid proteins on phage surface. Libraries of phage-displayed peptides may be screened for binding to specific ligands; determination of the gene sequence of the selected phage identifies the peptide sequence.

Polyhistidine-tag: Consists of approximately six histidine residues near the N- or C-terminus of a protein. The total number of histidine residues may vary. Polyhistidine tag fusion proteins bind to nickel and other metals and are eluted by the use of imidazole.

Post-translational modification: Modification of proteins following translation, including glycosylation, phosphorylation, sulfation, acetylation, and ribosylation.

Prenylation: The addition of a prenyl moiety to a protein. The addition of prenyl groups regulates protein-membrane interactions.

Primary antibody: An antibody generated against an antigenic target (a protein, peptide, carbohydrate, or other small molecule).

Protease: An enzyme that degrades proteins by hydrolyzing peptide bonds.

Proteasome: A large protein complex that degrades proteins that have been tagged for elimination, particularly those tagged by ubiquitination.

Protein domain: A structurally and functionally defined protein region. In proteins with multiple domains, the combination of the domains determines the function of the protein.

Protein fingerprint: The pattern of proteins in a cell or organism as determined by 2-D gel electrophoresis.

Proteome: The dynamic protein complement of an organism, including all post-translational modifications and protein interactions.

Pull-down Assay: An affinity chromatography method that involves using a tagged or labeled bait to create a specific affinity matrix that will enable binding and purification of a prey protein from a lysate sample or other protein-containing mixture.

Riboproteomics: The systematic characterization of RNA:protein interactions that affect the splicing, transport, lifetime and translation of RNAs.

Secondary antibody: An antibody that recognizes and binds a primary antibody. Secondary antibodies conjugated to enzymes and labels are key components of detection systems.

Shine-Dalgarno sequence: An mRNA sequence that precedes the translation initiation codon and is complementary to a ribosomal RNA.

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Signal peptidase: An endopeptidase that removes the signal peptide (signal sequence) following translocation of a protein.

Signal sequence: A short amino acid sequence that determines the localization of a protein within the cell.

Structural proteomics: Large scale determination of protein structures in three-dimensional space. Common methods are x-ray crystallography and NMR spectroscopy.

Two-dimensional electrophoresis (2-D gel): A technique used for the separation of complex protein mixtures. Proteins are separated in the first dimension on an isoelectric focusing gel, then separated by molecular weight using standard gel electrophoresis.

Ubiquitin: A protein that is covalently attached to lysine residues of other proteins, tagging them for proteolysis within proteasomes. Multiple ubiquitin units may be ligated to the protein, forming a multi-ubiquitin chain.

Western blot: A technique for the separation, immobilization, and detection of proteins, usually by a labeled antibody. Proteins are separated by gel electrophoresis, transferred to a membrane, and then probed with antibodies, which are then detected by chemiluminescent or colorimetric methods.

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Related Promega Products

Chapter 2

Product	Cat.#
CheckMate™ Flexi Mammalian Two-Hybrid System	C9360
CheckMate™ Mammalian Two-Hybrid System	E2440

Chapter 3

Product	Cat.#
TNT® T7 Quick Coupled Transcription/Translation System	L1171 L1170
TNT® SP6 Quick Coupled Transcription/Translation System	L2080 L2081
TNT® SP6 High Yield Protein Expression System	L3260 L3261
TNT® SP6 Coupled Reticulocyte Lysate System	L4600 L4601
TNT® T7 Coupled Reticulocyte Lysate System	L4610 L4611
TNT® T3 Coupled Reticulocyte Lysate System	L4950
TNT® T3 Coupled Wheat Germ Extract System	L4110
TNT® SP6 Coupled Wheat Germ Extract System	L4120
TNT® T7 Coupled Wheat Germ Extract System	L4130
HaloTag® pHT2 Vector	G8241
pFC8A (HaloTag®) Flexi® CMV Vector	C3631
pFC8K (HaloTag®) Flexi® CMV Vector	C3641
HaloLink™ Resin	G1911 G1912
HaloLink™ Magnetic Beads	G9311
MagneGST™ Pulldown System	V8870
MagneGST™ Protein Purification System	V8600 V8603
MagneGST™ Glutathione Particles	V8611 V8612
MagneHis™ Protein Purification System	V8500 V8550
MagneHis™ Ni-Particles	V8560 V8565
Single Step (KRX) Competent Cells	L3001 L3002

Chapter 4

Product	Cat.#
TNT® T7 Quick Coupled Transcription/Translation System	L1171 L1170
TNT® SP6 Quick Coupled Transcription/Translation System	L2080 L2081
TNT® SP6 High Yield Protein Expression System	L3260 L3261
TNT® SP6 Coupled Reticulocyte Lysate System	L4600 L4601
TNT® T7 Coupled Reticulocyte Lysate System	L4610 L4611
TNT® T3 Coupled Reticulocyte Lysate System	L4950
TNT® T3 Coupled Wheat Germ Extract System	L4110
TNT® SP6 Coupled Wheat Germ Extract System	L4120
TNT® T7 Coupled Wheat Germ Extract System	L4130

Chapter 6

Product	Cat.#
HaloCHIP™ System	G9410
HaloTag® pHT2 Vector	G8241
Anti-HaloTag® pAb	G9281

Chapter 7

Product	Cat.#
TNT® T7 Quick Coupled Transcription/Translation System	L1171 L1170
TNT® SP6 Quick Coupled Transcription/Translation System	L2080 L2081
TNT® SP6 High Yield Protein Expression System	L3260 L3261
TNT® SP6 Coupled Reticulocyte Lysate System	L4600 L4601
TNT® T7 Coupled Reticulocyte Lysate System	L4610 L4611
TNT® T3 Coupled Reticulocyte Lysate System	L4950
TNT® T3 Coupled Wheat Germ Extract System	L4110
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