Cell-Free Assays

Assay	Methodology
Enzymatic assays	
Intended Purpose: Determination of kinetic parameters such as Km, Vmax, etc.; IC50; Low, Medium, and High throughput Screening.	
Enzymatic assays for:	Abs, FI, FP, TRF, and more..
Kinases (PKC alpha)	TRF
Proteases (HIV1 Protease, Cathepsin B)	FRET, IQF
Phosphodieasterases (PDE V)	FP
Metabolic Enzymes	Abs
ECM (Collagenase, Transglutaminase)	
G-protein	FP
Adenylate Cyclase	
FAAH	FI
Biliverdin reductase	FI
Receptor activities	
Intended purpose: Ligand, agonist and antagonist binding: Determination of IC50 for inhibitors.	
Receptors	
Trimeric G-proteins	FP, TRF
Small G-proteins; Examples: Protein Gq, Rac	FP
Estrogen Receptors	FP
Immuno Assays	
hCGbeta, hFc, MCP1, Insulin	HRP
Collagen	FP
Proteomics	
Intended purpose: establishing assays for protein-protein interactions, preparation of samples, antibodies characterization	
Cell extractions	RIPA
Protein detection	SDS-PAGE, WB
Protein quantification	Bradfoed, Lawry, BCA
Proteomic profiling	Chemiluminescence
Protein-Protein interactions	
Example I	ELISA
Example II	FRET-TRF
Ligand protein interaction	
Phosphorylation & De-Phosphorylationin vitro	According to chosen assay
Detection of antigens in samples	ELISA, W.B.
Binding assays	IP&W.B., FRET, HTRF, alpha screen
Competition-based binding assays	IP&W.B., FRET, HTRF, alpha screen
Inhibition in binding assays	FRET, HTRF…
Detection of: cytoplasmic, nuclear, membrane, and cytoskeleton proteins	ELISA, W.B., FACS, IP
Fluorophore labeling of proteins and peptides	
Labeling proteins purification on PD10	Fluorescein, Biotin
Labeling peptides purification by CRO	Alexa-fluor
Western Blotting	
Preparing Samples for Western Blotting	
Preparing Samples for Quantitative Western Blotting	
Tricine SDS Gels	
Electrophoretic separation	
Electrophoretic Separation for Quantitative Western Blotting	
Transfer: iBlot, dry/semi-dry/wet transfer	
Quantitative Western Blotting	
Developing Western Blots Using the ECL, Chromogenic Methods	
Developing Western Blots with Scientific Imaging Film	
Visualization of Western Blots on the Kodak Image Station	
	Absorbance = Abs, Fluorescence= FI, Fluorescence Polarization = FP, Timer ressolved Fluorescence = TRF, FRET= Free ressonance Energy Transfer
Peptide/Compound Charaterization	
Solubility	Abs
Fluorescence Spectroscopy	3D FI
Protein - Peptide/Nucleic acids/Small molecule Interaction	FP ELISA
Nucleic acids quantification	FP
Biophysical assays	
Protein-small molecule	Thermal shift
Other	
In-vitro transcription/translation	
FRET in confocal microscopy	

Cell-Based Assays

Assay / Activity	Methodology
Tissue Culture	
P2 facility enable diverse tissue culture activities	
Grow and store Cells	Tissue culture methods
Cell bank storage available	
Growing, freezing, thawing, and packaging different cell lines	Tissue culture methods
Cell extractions and protein detection: preparing protein whole-cell extracts for quantitative gel analysis	See: Cell Free assays
Immunological assays	
Isolation of PBMCs from whole blood	Gravity Sedimentation
T Cell enrichment	Magnetic separation
T Cell Activation/Expansion	CD3+CD28 antibodies
Inflamation assays	
Potency Assays	
Proliferation/Activation/Apoptosis	MTT, XTT, WST1, WST3, IL2 (T cells),
Example: proliferation of lymph-node cells	BRDU
Toxicity assays	WST1, MTT, XTT, Anexcin CY3,Cell titer glo…
Cell Viability, cytotoxicity, anchorage-dependent cell death, and cellular senesc	Cell Biolabs kits
Angiogenesis Assay	use ECM matrix gel
Autophagy-a lysosomal degradation pathway for cytoplasmic material and is activated during cellular stress.	Two forms of LC3 may be seen by Western blot
Cell Adhesion and attachment	CytoSelect™ ECM Cell Adhesion Assays (Cell Biolabs
Example: Fibroblast attachment assay	
Cell migration: Chemotaxis, Haptotaxis, Transmigration, Wound healing	Cell Biolabs kits
Example: Transwell using mcp1 as chemo-attractent	Matrigel, Transwell + WST1
Cell activation & proliferation	
Interleukins, TNF, growth factors…	
Induction of cell differentiation (Such as L1 Pre- Adipocytes to differentiated Ad	Induction methods
Glucose Uptake in 3T3-L1	
Proliferation of fibroblasts (CRL2522) and endothelial cells treated with recombinant human proteins	
Binding to folate receptor in KB cells	
Cell signaling	Cell Biolabs kits
Trimeric G protein via forskolin induced cAMP	
Detect cell surface receptors and antigens	FACS, ELISA
Cell cycle	Propidium Iodide (PI) read in FACS
Ectopic expression of Proteins in Bacteria & mamalian cell lines	Transfections, Transformations
Cell activation measured by Ca++ influx	
Tgase activity	elisa
Gene expression/quantification	mRNA, RNA, miRNA, siRNA, DNA and RNA-Plex
Protein-Protein interaction	
mammalian two hybrid	
Binding Assays	IP-WB, ELISA
Other	
Confocal microscopy	
Lentiviral particales	

Bio-Analysis	
Assay / Activity	Methodology
Detection of peptides in serum	
Specific Assays	
Insulin, Calcitonin	Absorbance following HRP
Detection of antibodies in serum	
Intended purpose: Ligand, agonist and antagonist binding: Determination of IC50 for inhibitors.	
Specific antibodies	
human Fc from conditioned medium	HRP
Detection of Cytokines in serum	
Specific proteins	
Cytokines	HRP
Penetration of Blood Brain Barrier	
Extraction of evans Blue/ Fluorescein and labeled proteins from rat brain	FI/ Abs
	Absorbance = Abs, Fluorescence= FI, Fluorescence Polarization = FP, Timer ressolved Fluorescence = TRF, FRET= Free ressonance Energy Transfer

Early in vitro ADMETox	
Assay	Methodology
Absorption	
Intestinal absorption	Caco2 cells
Distribution	
Binding to plasma proteins	FP
Metabolism	
Metabolic Stability	microsomes, hepatocytes
Esterases metabolism	microsomes
CYP-Inhibition	recombinant CYP450 isozymes
CYP-Induction	mRNA
Toxicity	
Cytotoxicity	WST1-viability assay
Cardiotoxicity	Binding assay-FP