Sample Preparation for Protein Profiling

Protein Profiling Approach	Sample amount	Sample type*	Tolerated buffer reagents						Commonly used buffer for analysis
			Protease inhibitors	ionic detergents (e.g. SDS)	non-ionic detergents (e.g chaps, Triton X100)	reducing reagents	denaturants (e.g. urea, GuHCL)	high salt	
DIGE	50 to 100µg per sample	biological fluids, PBS washed cell pellets	yes	less than 1%	Triton X100 less than 1%	up to 2mg/ml DTT	yes	less than 50 mM, no primary amines	7M urea, 2M Thiourea, 4% CHAPS, 30mM Tris pH 8.8
iTRAQ	400µg total per experiment. For a 4plex, 100µg per sample; 8 plex, 25µg per sample	biological fluids, PBS washed cell pellets	no	yes <0.1%	yes <0.1%	no	<1M	no salt, no primary amines	8M Urea, 0.5M triethylammonium bicarbonate
2DLC	200µg to 5 mg	biological fluids, PBS washed cell pellets	yes	no	yes	yes	yes	no	7.5M urea, 2.5M Thiourea, 50 mM Tris, 2.5% octylglucoside, 6.25mM TCEP, 1.25mM protease inhibitor
ICAT	100µg each sample	biological fluids, PBS washed cell pellets	no	yes 0.1%	no	no	no	no	50mM Tris, 0.1% SDS
SILAC	50 to 100µg per sample	cells	no	no	no	yes	yes	no	8M urea, 50mM ammonium bicarbonate
MudPIT	5 to 100µg	biological fluids, PBS washed cell pellets	no	no	no	yes	yes	no	8M urea, 50mM ammonium bicarbonate
Label Free	0.2µg per run; recommend 5 to 20µg to start	biological fluids, PBS washed cell pellets	no	no	no	yes	yes	no	8M urea, 50mM ammonium bicarbonate

* biological fluids= serum/plasma, urine, CSF, lavage