

# Chemical Triphosphorylation of Oligonucleotides

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## Materials

Name	Company	Catalog Number	Comments
0.22 µm polyethersulfone syringe filter	MilliporeSigma	SLMP025SS	Syringe filter for removing crushed polyacrylamide gel particles (Section 5)
0.22 µm PTFE syringe filter	MilliporeSigma	SLLG013SL	Syringe filter for removing CPG resin (Section 5)
1 mL plastic syringes	ThermoFisher Scientific	14-823-434 (BD 309659)	Components of triphosphorylation apparatus (sections 2–4)
1,4-Dioxane, anhydrous	MilliporeSigma	296309	Triphosphorylation solvent (sections 2–4)
2-Chloro-4H-1,3,2-benzodioxaphosphorin-4-one, Salicyl Phosphorochloridite (SaPCI)	MilliporeSigma	324124	Triphosphorylation reagent (sections 2–4)
30 mL glass bottles	MilliporeSigma	23232	Bottles for preparing triphosphorylation solvents and TBAP solution (section 2)
3-way Stopcock, polycarbonate/polypropylene	Bio-Rad Laboratories	7328103	Component of triphosphorylation apparatus (sections 2–4)
40% acrylamide/bis-acrylamide solution, 19:1	Bio-Rad Laboratories	1610144	For PAGE (sections 5–7)
Acetonitrile, anhydrous, 100 mL	Glen Research	40-4050-50	Triphosphorylation solvent (sections 2–4)
Ammonia-neutralizing Trap	ThermoFisher Scientific	ANT100 and ANS121	For use with Speedvac DNA130 (section 5)
Ammonium persulfate (APS)	Bio-Rad Laboratories	1610700	For PAGE, catalyst for acrylamide polymerization (sections 5–7)
Aqueous ammonia, 28%	MilliporeSigma	338818	For preparing AMA deprotection reagent (section 5)
Aqueous methylamine, 40%	TCI America	TCI-M0137	For preparing AMA deprotection reagent (section 5)
Automated DNA/RNA oligonucleotide synthesizer	PerSeptive Biosystems	Expedite 8909 DNA/RNA Synthesizer	any column-based synthesizer is acceptable (section 1)
Bead-capture magnet	ThermoFisher Scientific	12320D	For streptavidin bead capture (section 7)
Bromophenol blue	Bio-Rad Laboratories	1610404	For PAGE urea loading buffer (section 5)
Deep vacuum oil pump	ThermoFisher Scientific	VLP200-115	For use with lyophilizer (section 5)

Drierite dessicant, 10-20 mesh	MilliporeSigma	737828	Dessicant for storing triphosphorylation chemicals and equipment (sections 1–2)
D-RNA 27.3t cross-chiral polymerase	prepared in house <sup>18</sup>	5'-GGUGGUGGAC GUGAUCAUUA CGGAUCACUA ACUCGUCAGU GCAUUGAGAA GGAGAAUAAA AUGCACAUAG GUCGAAAGAC CUUUAACAAG AACUGUAUCA CCGGAGGGCG AGCACCACC-3'	For cross-chiral ribozyme reactions (section 7)
D-RNA CPG solid supports, 1,000Å, prepackaged 1 µmole synthesis columns	Glen Research	20-3404-41E, 20-3415-41E, 20-3424-41E, 20-3430-41E	representative, for D-RNA oligonucleotide synthesis (section 1)
D-RNA TOM-protected phosphoramidites	ChemGenes	ANP-3201, 3202, 3203, 3205	representative, for D-RNA oligonucleotide synthesis (section 1)
Empty Expedite Synthesis Columns, 1µm	Glen Research	20-0021-01	Synthesis columns for use with Expedite DNA/RNA synthesizer (section 1)
EPDS, N-(2-Hydroxyethyl)piperazine-N'-(3-propanesulfonic acid), solid	MilliporeSigma	E1894	Ribozyme reaction buffer component (section 6)
Ethylenediaminetetraacetic acid (EDTA), solid	MilliporeSigma	EDS	Divalent metal ion chelator for use in various buffers (sections 5–7)
Filters for Expedite synthesis columns	Glen Research	20-0021-0F	Expedite-style synthesis column filters, for use with empty synthesis columns (section 1)
Fluorescent/phosphorescent gel scanner	Cytiva	Amersham Typhoon RGB, 29187193	For visualizing analytical PAGE (sections 6–7)
Formamide, deionized	VWR Life Science	97062	For PAGE formamide gel loading buffer (sections 6–7)
Gel image quantitation software	Cytiva	ImageQuant TL	For quantifying scanned gel images (section 6)
Glass desiccator	MilliporeSigma	CLS3121150	Triphosphorylation solvent storage (section 2)
L-RNA CPG solid supports, 1,000Å, bulk	ChemGenes	N-4691-10, N-4692-10, N-4693-10, N-4694-10	L-RNA oligonucleotide synthesis (section 1)
L-RNA hammerhead template	prepared in house <sup>18</sup>	5'-GCGCCUCAUC AGUCGAGCC-3'	For cross-chiral ribozyme reactions (section 7)
L-RNA primer	prepared in house <sup>18</sup>	5'-fluorescein-GGCUCGA-3'	For cross-chiral ribozyme reactions (section 7)
L-RNA TOM-protected phosphoramidites	ChemGenes	OP ANP-5201, 5202, 5203, 5205	L-RNA oligonucleotide synthesis (section 1)
Lyophilizer/Freeze Dryer	VirTis	Benchtop K	For concentrating oligonucleotides (section 5)
Magnesium Chloride Hexahydrate, solid	MilliporeSigma	M2670	For ribozyme reactions (sections 6–7)
N,N-Dimethylformamide, anhydrous	MilliporeSigma	227056	Triphosphorylation solvent (section 2)
NAP-25 Desalting column (Sephadex G-25 resin)	ThermoFisher Scientific	45000150	Disposable gravity-flow size exclusion chromatography columns containing Sephadex G-25 resin (section 5)
Non-coring stainless steel needle, 20 G	ThermoFisher Scientific	14-815-410	Needles for piercing rubber septa (sections 2–4)
Oligonucleotide extinction coefficient calculator	Integrated DNA Technologies	OligoAnalyzer Tool	Nearest-Neighbor Model Short Oligonucleotide 260nm extinction coefficient calculator (section 5)
Oxidizer solution, 0.1 M Iodine in THF/pyridine/water	ChemGenes	RN-1456	Triphosphorylation reagent (section 4)
PAGE plates	Timberlock/CBS	NGP-250-BO and NO	For PAGE (sections 5–7)
PAGE power supply	Bio-Rad Laboratories	PowerPac HV 1645056	For PAGE (sections 5–7)

PAGE spacers and combs (analytical)	Timberrock/CBS	VGS-0725 and VGC-0714	For PAGE (sections 6–7)
PAGE spacers and combs (preparative)	Timberrock/CBS	VGS-3025R and VGC-3001	For PAGE (section 5)
PAGE stand	Timberrock/CBS	ASG-250	For PAGE (sections 5–7)
Parafilm M	ThermoFisher Scientific	13-374-12 (Bemis PM999)	Wax sealing film for triphosphorylation apparatus (sections 2–4)
PCR thermocycler	Bio-Rad Laboratories	C1000 Touch Thermalcycler	For cross-chiral ribozyme reactions (section 7)
PD 10 Desalting column (Sephadex G-10 resin)	MilliporeSigma	GE17-0010-01	Disposable gravity-flow size exclusion chromatography columns containing Sephadex G-10 resin, for oligonucleotides < 15 nt (section 5)
Phosphor screens	Cytiva	28956480	For visualizing <sup>32</sup> P-labeled RNA (section 6)
Phosphoramidite synthesis reagents	Glen Research	30-3142-52, 40-4050-53, 40-4012-52, 40-4122-52, 40-4132-52, 40-4060-62	representative, for standard RNA/DNA synthesis (section 1)
Polypropylene screw-cap sealable tube	MilliporeSigma	BR780752	1.5 mL microcentrifuge tubes with screw-cap and silicone O-ring, for safe AMA deprotection (section 5)
Pyridine, anhydrous	MilliporeSigma	270970	Triphosphorylation solvent (section 2)
Reverse-phase liquid chromatography/electrospray ionization mass spectrometry (RP-LC/ESI-MS)	Novatia	n/a	Commercial service for LC/MS specializing in oligonucleotides (section 5)
Rubber Septa (ID x OD 7.9 mm x 14 mm), white	MilliporeSigma	Z564702	Septa for preparing triphosphorylation solvents and TBAP (section 2)
Self-replicator ribozyme E	prepared in house <sup>14</sup>	5'-GGAAGUUGUG CUCGAUUGUU ACGUAAGUAA CAGUUUGAAU GGUUGAAGUA UGAGACCGCA ACUUA-3'	For self-replicator ribozyme reactions (section 6)
Self-replicator substrate A	prepared in house <sup>14</sup>	5'- <sup>32</sup> P-GGAAGUUGUG CUCGAUUGUU ACGUAAGUAA CAGUUUGAAU GGUUGAAGUA U-3'-OH	For self-replicator ribozyme reactions (section 6)
Self-replicator substrate B, transcribed	prepared in house <sup>14</sup>	5'-pppGAGACCGCAA CUUA-3'	For self-replicator ribozyme reactions (section 6)
Small Drying Traps, 4 Å molecular sieves	ChemGenes	DMT-1975	Drying traps for DNA/RNA synthesizer phosphoramidites and triphosphorylation reagents (sections 1–2)
Sodium Chloride (NaCl), solid	MilliporeSigma	S7653	Salt for use in various buffers (sections 5–7)
Sodium Hydroxide (NaOH), solid	MilliporeSigma	S8045	Salt for use in various buffers (sections 5–7)
Statistical data-fitting software	GraphPad	Prism	For fitting data from analytical PAGE to kinetic models (section 6)
Streptavidin-coated magnetic beads	ThermoFisher Scientific	65002	For capturing biotin-labeled RNA in cross-chiral ribozyme reactions (section 7)
Sucrose	MilliporeSigma	84097	For PAGE urea loading buffer (section 5)
TBE running buffer, 10x	ThermoFisher Scientific	AAJ62788K3	For PAGE (sections 5–7)
Tetrabutylammonium Fluoride, 1.0 M solution in Tetrahydrofuran	Aldrich	216143	For removing 2'-silyl protecting groups (section 5)

Tetramethylethylenediamine (TEMED)	Bio-Rad Laboratories	1610801	For polymerizing acrylamide for PAGE (sections 5–7)
Tributylamine	MilliporeSigma	90781	Triphosphorylation reagent (section 2)
Tributylammonium pyrophosphate (TBAP)	MilliporeSigma	P8533	Triphosphorylation reagent (section 2)
Tris base	MilliporeSigma	T6666	Buffering agent for use in various buffers (sections 5–7)
TWEEN20 polysorbate detergent	MilliporeSigma	P7949	Neutral detergent for use with magnetic beads (Section 7)
Urea	MilliporeSigma	U5378	For PAGE and gel loading buffer (sections 5–7)
UV-Vis spectrophotometer	ThermoFisher Scientific	NanoDrop 2000, ND2000	For measuring oligonucleotide concentrations (section 5)
Vacuum centrifuge	ThermoFisher Scientific	Savant Speedvac DNA130-115 Vacuum Concentrator	For removing AMA and THF (section 5)
Xylene cyanol	Bio-Rad Laboratories	1610423	For PAGE urea loading buffer (section 5)