P1156B HiPure Plasmid EF Maxi Kit B Performance Validation Report

Low-Endotoxin Maxi-Format Plasmid DNA Purification

Report Overview

This report summarizes internal validation experiments for the P1156B HiPure Plasmid EF Maxi Kit B. P1156B is an upgraded low-endotoxin Maxi-format plasmid purification workflow based on the RC7 column format, optimized endotoxin-removal chemistry and flexible centrifugation or vacuum processing.

The validation focused on plasmid yield, purity, operation mode comparison and ethanol carryover control. pcDNA3.1-containing bacterial cultures were processed at 50 mL, 100 mL and 200 mL input volumes. Purified plasmid DNA was evaluated by NanoDrop measurement.

Experiment 1. Comparison of Centrifugation and Vacuum Processing

Objective	To compare plasmid extraction performance using centrifugation and vacuum-based processing with the P1156B RC7 Maxi column workflow.
Sample	LB bacterial culture containing pcDNA3.1 vector, cultured for 14 hours. Input volumes: 50 mL, 100 mL and 200 mL.
Workflow summary	Bacterial cells were collected by centrifugation, resuspended in Buffer P1/RNase A, lysed with Buffer P2, neutralized with Buffer NS3, clarified by centrifugation and filtration, treated with Buffer ER2, mixed with isopropanol, and loaded onto the RC7 Maxi column by either centrifugation or vacuum processing.
Evaluation	Actual elution volume, plasmid concentration, A260/280, A260/230 and total plasmid yield were recorded.

Table 1. Plasmid yield and purity using centrifugation and vacuum processing

Culture Volume	Processing Mode	Elution Step	Actual Elution Volume (μL)	Conc. (ng/μL)	A260/280	A260/230	Yield (μg)
50 mL	Centrifugation	1st, 0.7 mL	550	605.5	1.94	2.30	333
		2nd, 0.7 mL	685	102.9	1.93	2.03	71
		Combined	1235	327.2	1.93	2.24	404
		1st, 0.7 mL	520	597.5	1.93	2.25	311
		2nd, 0.7 mL	700	117.9	1.89	2.06	83
		Combined	1220	332.1	1.92	2.21	405
	Vacuum filtration	1st, 0.7 mL	550	597.7	1.94	2.27	329
		2nd, 0.7 mL	690	116.8	1.91	2.02	81
		Combined	1240	334.3	1.93	2.21	414
		1st, 0.7 mL	525	619.2	1.95	2.30	325
		2nd, 0.7 mL	700	128.3	1.91	2.14	90
		Combined	1225	339.0	1.94	2.26	415
100 mL	Centrifugation	1st, 0.8 mL	630	772.1	1.91	2.26	486
		2nd, 0.8 mL	790	189.6	1.91	2.16	150
		Combined	1420	454.9	1.93	2.26	646
		1st, 0.8 mL	620	931.4	1.92	2.26	577
		2nd, 0.8 mL	790	189.9	1.90	2.06	150
		Combined	1410	525.8	1.93	2.24	741

Culture Volume	Processing Mode	Elution Step	Actual Elution Volume (μL)	Conc. (ng/μL)	A260/280	A260/230	Yield (μg)
	Vacuum filtration	1st, 0.8 mL	600	953.1	1.94	2.30	572
		2nd, 0.8 mL	800	225.9	1.90	2.15	181
		Combined	1400	544.9	1.93	2.26	763
		1st, 0.8 mL	580	1059.9	1.94	2.28	615
		2nd, 0.8 mL	800	179.0	1.91	2.09	143
		Combined	1380	561.5	1.93	2.23	775
200 mL	Centrifugation	1st, 1.0 mL	740	1282.0	1.93	2.29	949
		2nd, 1.0 mL	1020	362.3	1.91	2.13	370
		Combined	1760	739.7	1.92	2.27	1302
		1st, 1.0 mL	720	1502.6	1.93	2.27	1082
		2nd, 1.0 mL	1030	319.9	1.91	2.14	329
		Combined	1750	795.8	1.94	2.28	1393
	Vacuum filtration	1st, 1.0 mL	730	1437.9	1.93	2.29	1050
		2nd, 1.0 mL	1020	330.8	1.91	2.15	337
		Combined	1750	786.3	1.94	2.28	1376
		1st, 1.0 mL	710	1453.9	1.94	2.25	1032
		2nd, 1.0 mL	1010	530.2	1.93	2.21	536
		Combined	1720	897.3	1.94	2.26	1543

Experiment 1 Summary

Both centrifugation and vacuum processing produced high-quality plasmid DNA across the tested culture volumes. A260/280 values were approximately 1.89-1.95, and A260/230 values were approximately 2.02-2.30 across the tested conditions.

Plasmid yield increased with culture input. Combined yields were approximately 404-415 μg from 50 mL culture, 646-775 μg from 100 mL culture and 1302-1543 μg from 200 mL culture under the tested conditions.

Centrifugation and vacuum processing showed comparable recovery profiles, supporting flexible use of the P1156B workflow in laboratories equipped with either standard centrifuges or vacuum filtration devices.

Experiment 2. Ethanol Carryover and Drying Evaluation of the RC7 Column

The second experiment evaluated residual PW2 wash buffer/ethanol after column washing and drying. The RC7 column was weighed after PW2 washing, after spin drying and after additional room-temperature drying or heat drying. Increased column weight was used as an indicator of residual liquid.

Two operation strategies were compared: (1) a wash centrifugation step followed by an additional spin-drying step, and (2) a combined centrifugation/drying step.

Table 2. RC7 column residual liquid evaluation after PW2 washing and drying

Operation	Column Weight Increase After PW2 Wash	Operation	Column Weight Increase After Spin Drying	Dry at Room Temperature for 10 min	Addition Dry at Room Temperature for 10 min
Add 9 mL Buffer PW2; centrifuge at 8000 rpm for 3 min	100 mg	Centrifuge at 8000 rpm spin for 10 min	38 mg	12 mg	6 mg
Add 9 mL Buffer PW2; centrifuge at 8000 rpm for 3 min	100 mg	Centrifuge at 8000 rpm spin for 10 min	26 mg	15 mg	10 mg
Add 9 mL Buffer PW2; centrifuge at 8000 rpm for 10 min			59 mg	20 mg	10 mg
Add 9 mL Buffer PW2; centrifuge at 8000 rpm for 10 min			59 mg	20 mg	10 mg

Experiment 2 Summary

The RC7 Maxi column workflow can combine centrifugation and drying into a simplified operation step. After PW2 washing followed by 8000 rpm centrifugation for 10 minutes, the column weight increase was approximately 59-60 mg, corresponding to about 60 µL of residual PW2 wash buffer.

To further reduce ethanol carryover, the column should be air-dried at room temperature for 10-20 minutes after spin drying. Under the tested conditions, this reduced residual ethanol to below approximately 10 µL, supporting downstream applications that are sensitive to ethanol carryover.

Overall Conclusion

The P1156B HiPure Plasmid EF Maxi Kit B demonstrated stable plasmid recovery and purity across 50-200 mL pcDNA3.1 bacterial culture inputs. The RC7 column format supported both centrifugation and vacuum-based processing with comparable yield profiles.

The validation data support the use of P1156B as an upgraded low-endotoxin Maxi-format plasmid purification workflow for laboratories requiring flexible operation, high plasmid recovery and controlled ethanol removal before downstream applications.

Note: This validation report summarizes internal test results under the specified conditions. Actual plasmid yield may vary depending on plasmid copy number, bacterial strain, culture density, culture condition and elution setup.