NucleoBond® RS purification workflow

Protocol at a glance (Rev. 05)

	RS 10	RS 50	RS 100	RS 200
1 – 3 Cell cultivation and harvest	4 °C, 15 min 4,500 – 6,000 × *g*			
	max. recom: ODV: 3,000 CDW: 8 g typ. Yield: ~10 mg	max. recom: ODV: 15,000 CDW: 40 g typ. Yield: ~50 mg	max. recom: ODV: 30,000 CDW: 80 g typ. Yield: ~100 mg	max. recom: ODV: 54,000 CDW: 145 g typ. Yield: ~190 mg
4 – 5 Cell lysis *(Important: Check Buffer LYS-EF for precipitated SDS)*	60 mL Buffer RES-EF 60 mL Buffer LYS-EF	300 mL Buffer RES-EF 300 mL Buffer LYS-EF	600 mL Buffer RES-EF 600 mL Buffer LYS-EF	1080 mL Buffer RES-EF 1080 mL Buffer LYS-EF
	RT, max 5 min			
6 Neutralization	60 mL Buffer NEU-EF	300 mL Buffer NEU-EF	600 mL Buffer NEU-EF	1080 mL Buffer NEU-EF
	Mix thoroughly until colorless Incubate 15 min on ice!			
Maximum recommended pump speed	5 mL/min	10 mL/min	10 mL/min	10 mL/min
7 Equilibration of the column	30 mL EQ-EF	120 mL EQ-EF	250 mL EQ-EF	400 mL EQ-EF
8 Clarification of the lysate	Clarify and filtrate the crude lysate to 0.45/0.22 µm			
9 Binding	Load cleared lysate on NucleoBond® RS column			
10 1st Wash	30 mL Buffer ENTO-EF	150 mL Buffer ENTO-EF	300 mL Buffer ENTO-EF	550 mL Buffer ENTO-EF
11 2nd Wash	30 mL Buffer WASH-EF	150 mL Buffer WASH-EF	300 mL Buffer WASH-EF	550 mL Buffer WASH-EF
13 Elution	30 mL Buffer ELU-EF	120 mL Buffer ELU-EF	250 mL Buffer ELU-EF	400 mL Buffer ELU-EF
14 Precipitation	21 mL Isopropanol	84 mL Isopropanol	175 mL Isopropanol	280 mL Isopropanol
	Mix thoroughly 4,500 – 15,000 × *g* 4 °C, 15 min			
15 Washing and drying	5 mL 70 % EtOH 4,500 – 15,000 × *g* RT, 5 min Approx. RT, 10 – 15 min	15 mL 70 % EtOH 4,500 – 15,000 × *g* RT, 5 min Approx. RT, 30 – 60 min	25 mL 70 % EtOH 4,500 – 15,000 × *g* RT, 5 min Approx. RT, 45 – 90 min	50 mL 70 % EtOH 4,500 – 15,000 × *g* RT, 5 min Approx. RT, 75 – 120 min
16 Reconstitution	Appropriate volume of TE-EF or H_2O-EF. It is recommended to choose the resuspension volume according to the requirements of the downstream application. (for var., @ 1 mg/mL)			
	8 – 10 mL	40 – 50 mL	80 – 100 mL	170 – 190 mL

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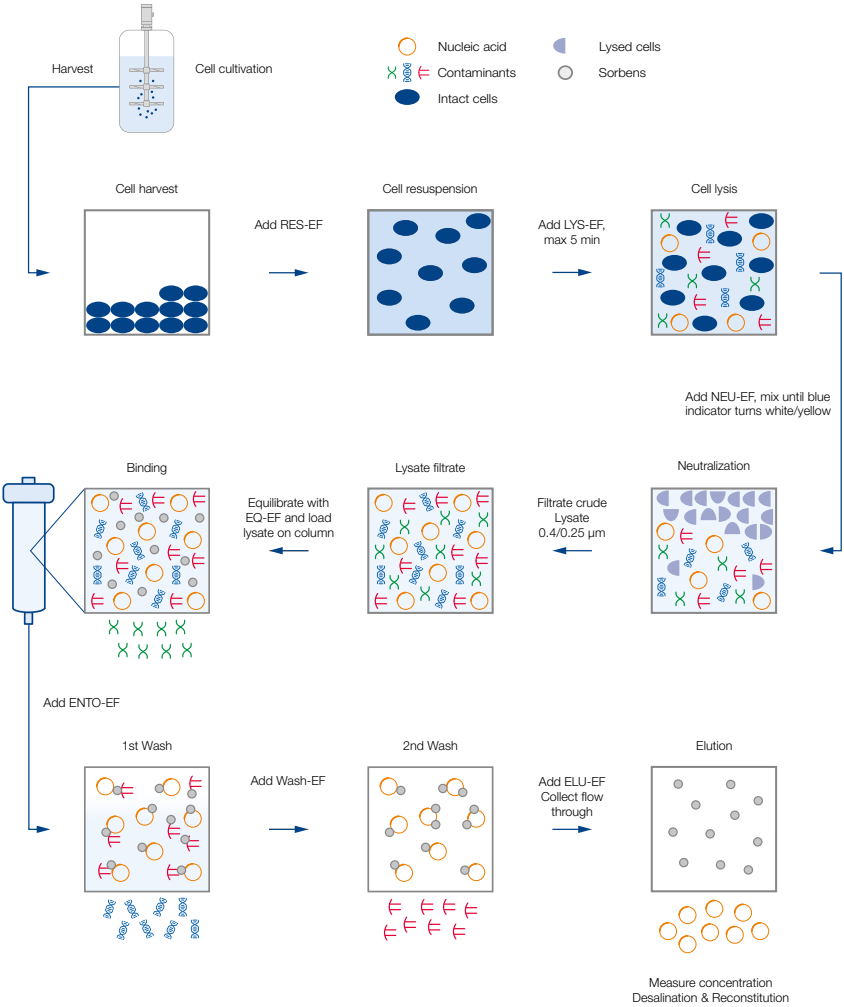

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NucleoBond® RS RNase free purification workflow

Protocol at a glance (Rev. 05)

	RS 10	RS 50	RS 100	RS 200
1 – 3 Cell cultivation and harvest	4 °C, 15 min 4,500 – 6,000 × *g*			
	max. recom: ODV: 3,000 CDW: 8 g typ. Yield: ~10 mg	max. recom: ODV: 15,000 CDW: 40 g typ. Yield: ~50 mg	max. recom: ODV: 30,000 CDW: 80 g typ. Yield: ~100 mg	max. recom: ODV: 54,000 CDW: 145 g typ. Yield: ~190 mg
4 – 5 Cell lysis *(Important: Check Buffer LYS-EF for precipitated SDS)*	60 mL Buffer RES-EF 60 mL Buffer LYS-EF	300 mL Buffer RES-EF 300 mL Buffer LYS-EF	600 mL Buffer RES-EF 600 mL Buffer LYS-EF	1080 mL Buffer RES-EF 1080 mL Buffer LYS-EF
	RT, max 5 min			
6 Neutralization	60 mL Buffer NEU-EF	300 mL Buffer NEU-EF	600 mL Buffer NEU-EF	1080 mL Buffer NEU-EF
	Mix thoroughly until colorless Incubate 15 min on ice!			
Maximum recommended pump speed	5 mL/min	10 mL/min	10 mL/min	10 mL/min
7 Equilibration of the column	30 mL EQ-EF	120 mL EQ-EF	250 mL EQ-EF	400 mL EQ-EF
8 Clarification of the lysate	Clarify the crude lysate (BottleTop Filter) or 1 – 5 µm (Capsule filter)			
9 RNA precipitation	Add 0.3 (v/v) isopropanol and mix thoroughly Incubate 15 min on ice!			
10 RNA removal	Clarify and filtrate the cloudy lysate to 0.45/0.22 µm			
11 DNA precipitation	Add 0.16 (v/v) isopropanol to the present lysate-isopropanol mixture volume.			
12 Pellet wash	Wash with 70 % Ethanol 4,500 – 15,000 × g RT, 5 min			
13 Reconstitution binding parameters	Reconstitution of pDNA with BMC-Buffer until pellet dissolved.			
14 Binding	Load RNA free lysate on NucleoBond® RS column			
15 1st Wash	75 mL Buffer ENTO-EF	375 mL Buffer ENTO-EF	750 mL Buffer ENTO-EF	1380 mL Buffer ENTO-EF
16 2nd Wash	75 mL Buffer WASH-E	375 mL Buffer WASH-EF	750 mL Buffer WASH-EF	1380 mL Buffer WASH-EF
17 Elution	30 mL Buffer ELU-EF	120 mL Buffer ELU-EF	250 mL Buffer ELU-EF	400 mL Buffer ELU-EF

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	RS 10	RS 50	RS 100	RS 200
18 Precipitation	21 mL Isopropanol	84 mL Isopropanol	175 mL Isopropanol	280 mL Isopropanol
	Mix thoroughly 4,500 – 15,000 × g 4 °C, 15 min			
19 Washing and drying	5 mL 70 % EtOH 4,500 – 15,000 × g RT, 5 min Approx. RT, 10 – 15 min	15 mL 70 % EtOH 4,500 – 15,000 × g RT, 5 min Approx. RT, 30 – 60 min	25 mL 70 % EtOH 4,500 – 15,000 × g RT, 5 min Approx. RT, 45 – 90 min	50 mL 70 % EtOH 4,500 – 15,000 × g RT, 5 min Approx. RT, 75 – 120 min
20 Reconstitution	Appropriate volume of TE-EF or H_2O-EF. It is recommended to choose the resuspension volume according to the requirements of the downstream application. (for var., @ 1 mg/mL)			
	8 – 10 mL	40 – 50 mL	80 – 100 mL	170 – 190 mL

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