ARROW Xpress Plasmid Kit

Cat. AXP002, AXP025
Qty. 2r, 25r

ARROWTEC

Content and Storage

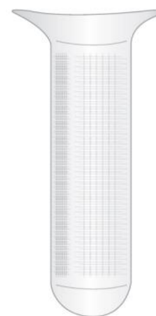
ARROW Xpress Plasmid Kit

AXP002 // AXP025

Store at room temperature 15℃~25℃

Component.	2 rxn Volume.	25 rxn Volume.
PX 1 buffer	25 ml	130 ml x 2
PX 2 buffer	25 ml	130 ml x 2
PX 3 buffer	25 ml	130 ml x 2
PEQ buffer	60 ml	200 ml x 4
PWA buffer	35 ml	200 ml x 2
PEL buffer	25 ml	130 ml x 2
RNase A (50 mg/ml)	--	520 µl
PMI Column	2 pcs	25 pcs
Thimble	2 pcs	25 pcs

PMI Column


Thimble

- Add provided RNaseA (260 µl) to PX1 Buffer (130 ml) and store at 2~8°C. The solution will be stable for at least 6 months .
- If precipitate has formed in PX 2 Buffer, warm the buffer in a 37°C water bath to dissolve.
- *Isopropanol and 70% ethonal are required.*

Description

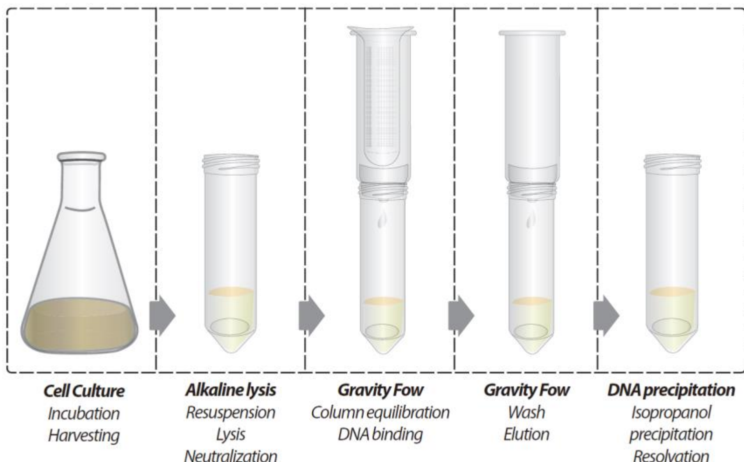
The ARROW Xpress plasmid Kit uses pre-packed anion exchange resin columns to obtain high purity plasmid DNA from100-400ml. In the process, the modified alkaline lysis method (1) and RNaseA treament are used to get cleared cell lysate with minimal genomic DNA and RNA contaminants. After plasmid DNA has been bound to the column, the contaminants can be washed off with Wash Buffer. Finally, the purified plasmid DNA is eluted by high salt buffer and then precipitated with isopropanol for desalting. The entire procedure can be comple--ted in 80 minutes and the resulting high purity plasmid DNA is suitable for transfection, sequencing reaction, PCR and in vitro transcription.

Recommended Culture Volume

	Rec. O.D V	$O.D_{600}$ = 2	$O.D_{600}$ = 4	DNA yield
High copy	200	100 ml	50 ml	≥ 500 µg
High copy	400	200 ml	100 ml	≥ 800 µg
Low copy	800	400 ml	200 ml	≥ 500 µg

$O.DV = O.D_{600}$ x Volume (ml)

Overview



Cell Culture
Incubation
Harvesting

Alkaline lysis
Resuspension
Lysis
Neutralization

Gravity Fow
Column equilibration
DNA binding

Gravity Fow
Wash
Elution

DNA precipitation
Isopropanol
precipitation
Resolvation

Standard Protocol

1 *Cell Harvesting*

Harvest the bacterial culture by centrifugation at 6,000 xg for 15 minutes at 4°C and discard the supernatant completely.

2 *Resuspension (PX 1 buffer)*

Apply 10ml of PX1 Buffer (RNase A added) to resuspend the cell pellet by vortex and pipetting.

3 *Cell Lysis (PX 2 buffer)*

Add 10 ml of PX2 Buffer and mix gently by inverting the tube 10-15 times.
Do not vortex, avoid shearing genomic DNA.
Stand for 5 minutes at room temperature until lysate clears.

4 *Equilibration (PEQ buffer)*

Place a PMI Column together with the Thimble on a new 50 ml centrifuge tube (not provided).
Equilibrate the Thimble by applying 20ml of PEQ Buffer, allow the Thimble to empty by gravity flow.

5 *Neutralization (PX 3 buffer)*

Add 10 ml of PX3 Buffer into the lysate and mix immediately by inverting the tube 10-15 times.
Do not vortex. Please incubate at room temperature for 5 minutes.

6 *Clarification and loading (DNA binding)*

Inverting the tube 3 times, before applying the lysate to the equilibrated Thimble to avoid clogging.
The lysate is simultaneously cleared and loaded onto the column with Thimble to avoid clogging.

Standard Protocol (continues)

7 *Rinse Thimble and PMI Column (PEQ Buffer)*

***R**inse the Thimble and PMI column with 10 ml of PEQ Buffer. Apply the buffer to the funnel shaped rim of the Thimble and make sure it is washing out the remained lysate.
***D**iscard the Thimble.

8 *Wash PMI Column (PWA Buffer)*

***W**ash the PMI column with 10 ml PWA Buffer, allow the PMI column to empty by gravity flow. It is important to remove the Thimble before this step to avoid low purity.

9 *Elution (PEL Buffer)*

***P**lace the PMI column in a clean 50 ml centrifuge tube (not provided) , add 10 ml of PEL Buffer to elute DNA by gravity flow.

10 *Precipitation*

***A**dd 0.75 volume of room-temperature isopropanol to precipitate the eluted plasmid DNA.(For example, add 7.5 ml of isopropanol to 10 ml of PEL Buffer)
***I**nverting 15 times or vortex (mix well) and stand for 2 min.
***C**entrifuge at 20,000 xg for 30 min at 4°C. Carefully discard the supernatant.

11 *Wash and dry DNA pellet*

***A**dd room-temperature 70% ethanol 5 ml to wash the pellet
***C**entrifuge at 20,000 xg for 10 min at room temperature. Carefully discard the supernatant
***A**llow the pellet to dry at room temperature for 10 min.

12 *Reconstitute DNA*

***D**issolve the DNA pellet in an appropriate volume of Buffer TE (not provided) or sterile H_2O.

Troubleshooting

Problem	Possitive cause and suggestions
No or low plasmid DNA yield	Plasmid did not propagate ▸ Check plasmid content in the cleared lysate. Use colonies from fresh plates for inoculation and add selective antibiotic to plates and media. Alkaline lysis was inefficient ▸ Too much cell mass was used. ▸ Check Buffer PM2 for SDS precipitation before use, especially after storage below 20 ° C. If necessary incubate the bottle for several minutes at 30 - 40 ° C and mix well until SDS is redissolved. Sample/lysate is too viscous ▸ Too much cell mass was used. ▸ Make sure to mix well after neutralization to completely precipitate SDS and chromosomal DNA. Otherwise, filtration efficiency and flow rate go down and SDS prevents DNA from binding to the column. pH or salt concentrations of buffers are too high ▸ Check plasmid content in the wash fractions. Keep all buffers tightly closed. ▸ Check and adjust pH of Buffer PWA (pH 7.0), and PEL (pH 8.5) with HCl or NaOH if necessary.
Thimble clogs during filtration	Culture volumes are too large ▸ Larger lysis buffer volumes. Precipitate was not resuspended before loading ▸ Invert crude lysate at least 3 times directly before loading. Incomplete precipitation step ▸ Make sure to mix well after neutralization to completely precipitate SDS and chromosomal DNA.
PMI Column is blocked or very slow	Sample is too viscous ▸ Do not attempt to purify lysate prepared from a culture volume larger than recommended for any given column size with standard lysis buffer volumes. Incomplete lysis not only blocks the column but can also significantly reduce yields. ▸ Make sure to mix well after neutralization to completely precipitate SDS and chromosomal DNA. Lysate was not cleared completely ▸ Thimbler or centrifuge at higher speed or for a longer period of time. ▸ Precipitates occur during storage. Clear lysate again before loading the column.
Genomic DNA contamination of plasmid DNA	Lysis treatment was too harsh ▸ Make sure not to lyse in Buffer PX2 for more than 5 min. Lysate was mixed too vigorously or vortexed after lysis ▸ Invert tube for only 5 times. Do not vortex after addition of Buffer PM2. ▸ Use larger tubes or reduce culture volumes for easier mixing.

Troubleshooting

Problem	Possitive cause and suggestions
RNA contamination of plasmid DNA	RNase digestion was inefficient ‣ RNase was not added to Buffer PEQ or stored improperly. Add new RNase to Buffer PEQ, and store at 4° C. pH or salt concentration of wash buffer is too low ‣ Check RNA content in the wash fractions. Keep all buffers tightly closed. Check pH of Buffer PWA (pH 7.0) and adjust with HCl or NaOH if necessary. Wash step with Buffer PEQ was not sufficient ‣ Double or triple washing step with Buffer PWAA. Additional Buffer PWA can be ordered separately.
Low purity (A260/A280 < 1.8)	Thimble was not removed before second washing step ‣ Protein content too high due to inefficient washing. Remove the thimble before performing the second washing step with Buffer PWA. Buffer PWA was used instead of Buffer PEQ for the first wash Buffer PEQ has to be used to wash out the thimble to avoid SDS carryover. Only minimal amounts of DNA were loaded onto the column Excess free binding capacity requires more extensive washing – double washing step with Buffer PWA. Reduce lysis time < 5 min.
Nucleic acid pellet is opaque or white instead of clear and glassy	Co-precipitation of salt ‣ Check isopropanol purity, and perform precipitation at room temperature (20 - 25 ° C) but centrifuge at 4 ° C. Do not let the eluate drip from the column into isopropanol but add isopropanol to the final eluate and mix immediately. Try resuspending the pellet in Buffer PWA, and reload onto the same PMI Column. Wash the column several times with Buffer PWA before loading.
Nucleic acid pellet does not resuspend in buffer	Pellet was over-dried ‣ Try to dissolve at higher temperatures for a longer period of time (e.g. 2 h at 37° C or overnight at RT), preferably under constant spinning (3D-shaker). Co-precipitation of salt or residual alcohol ‣ Wash the pellet again with 70 % ethanol, or increase the reconstitution buffer volume. Insoluble particles in redissolved DNA ‣ Centrifuge the redissolved DNA to pellet the insoluble particles and transfer supernatant to a new tube. Insoluble particles do not affect DNA quality.

Troubleshooting

Problem	Possitive cause and suggestions
Purified plasmid does not perform well in subsequent reactions	Plasmid DNA is contaminated with chromosomal DNA or RNA ► Refer to the detailed troubleshooting above. Plasmid DNA is contaminated with residual alcohol ► Plasmid DNA was not dried completely before redissolving. Precipitate DNA again by adding 1 / 10 volume of 3 M NaAc pH 5.0 and 0.7 volumes of isopropanol. DNA is degraded ► Make sure that your entire equipment (pipettes, centrifuge tubes, etc.) is clean and nuclease-free. ► Do not lyse the sample with Buffer PX2 for more than 5 min. DNA is irreversibly denatured ► A denatured plasmid band runs faster on the gel than the supercoiled conformation. Do not lyse the sample after addition of Buffer PX2 for more than 5 minutes.