PurMa Biologics, LLC

PurMaFectin™ Ultra
User Manual

Cat #: P3E111204-01 1X 1ml
Cat #: P3E111204-05 5X 1ml

PurMaFectin™ Ultra	
Catalog #	Volume
P3E111204-01	1X 1ml
P3E111204-05	5X 1ml

Protocol	
Time	Description
Day 0	- Seed the plates Cell 16 to 20 hours before transfection with around 60-70% confluency. - Change the medium 30 minutes before the transfection. - Presence of serum usually does not affect transfection efficiency, but we recommend using a medium without serum.
Day 1	- Mix the DNA by with medium, and vortex for 2 seconds and incubate 5 minutes. (See table 1 for the ration of DNA and the medium). - Please read the criteria of the DNA in the description section. Plasmid for transfection should relatively have an acceptable purity (A260/A280 ≥1.7). - Mix DNA with PurMaFectin™ Ultra and tap 1 time with the tip of finger. - Incubate at room Temperature for 25min. - Add mixtures into dish/plate containing the cells.
Day 2-3	- Most of the cells are transfected within the first six hours, but to gain the maximum results, we recommend removing the mixture after 24-48 hours. Primary cells usually need longer time. We recommend finding the best duration based on your cell line. - Aspirate the medium and refill the plate with fresh culture medium including FBS.

Table 1. PurMaFectin™ Ultra Transfection Volume and ratio Guide				
Culture Dish/Plate	Media Volume	Plasmid	Serum-Free Medium	UltraTRAX™
96-well	100 µL	250 ng	10 µL	0.75 µL
24-well	500 µL	500 ng	25 µL	1.5 µL
12-well	700 µL	750 ng	35 µL	2.25 µL
6-well	1 mL	1 µg	50 µL	3 µL
6 cm	3 mL	2.5 µg	150 µL	7.5 µL
10 cm	6 mL	5 µg	300 µL	15 µL

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Trouble Shooting		
Problem	**Cause**	**Solution**
Low transfection efficiency	DNA/ transfection reagent ratio NOT optimal for your cell line	Prepare complexes using a DNA (µg) to PurMaFectin™ Ultra (µl) of 1: 3. This ratio works for most of the cell line to start with. To reach the maximum capacity in your transfection fine optimization may be necessary for most cell lines.
Low cell viability following transfection	Plasmid DNA preparation contains high levels of endotoxin.	- Ensure that the plasmid DNA used for transfection is of high quality A260/A280 ≥ 1.7. - Use PurMa™ Endotoxin Elimination Kit (Cat# P6H1216251) (See the description for PurMaFectin™ Ultra, Cat: P3E111204)
Reduced cell viability following transfection	Antibacterial reagents were used in growth medium during transfection.	Do not use antibiotics in growth medium because during transfection, cells are more permeable to antibiotics, which may cause toxicity.
Transfect results not reproducible	Transfection is performed at different cell confluency, or at different Plasmid/ PurMaFectin™ Ultra ratios	Transfection performance reproducibility is dependent on: - Consistency in cell splitting, plating with the same number of trypsinized cells. - The same Ratio of DNA: transfection reagent ratios). - Preparation of the DNA - Do NOT switch from one media to another. - Perform an optimization for your transfection if you are using new plasmid, or new cell line and after optimization, DO NOT change the protocol.