PurMa Biologics, LLC

PurMaFectin™

Cat #: P3E010204-01 1X 1ml
Cat #: P3E010204-05 5X 1ml

PurMaPlus™

Cat #: P3E010205-01 1X 1ml
Cat #: P3E010205-05 5X 1ml

PurMaFectin™	
Catalog #	Volume
P3E212204-01	1X 1ml
P3E212204-05	5X 1ml

PurMaPlus™	
Catalog #	Volume
P3E212205-01	1X 1ml
P3E212205-05	5X 1ml

Table1. Protocol

Time	Description
Seeding	
Day 0	- Seed the plates 24 hours before the transfection to be 80-90% confluent on the time of transfection. - **To keep the consistency between various transfections, it is crucial to keep the same number of hours after the seeding and before the transfections as well as the number of trypsinized cells are being used in the in seeding.** - Do NOT use antibiotics in the medium. - Change the medium 4 hours before the transfection. - Presence of serum usually does not affect transfection efficiency, but we recommend using a medium without serum.
Preparation of PurMaFectin™ / DNA complex and the transfection procedures. The following procedures and amount of the reagents is to transfect a 96- well format. See Table 2 for other plate sizes.	
Day1	- Change the media 2-4 hours before transfection. - Add 0.2 µg DNA and 0.4 µl PlurMaPlus™ reagents to 25 µl of medium **without serum.** - Gently mix. Incubate for 5 minutes at room temperature. - Mix PurMaFectin™ reagent gently before use, then dilute 0.5 µl to 25 µl of medium without serum. - Incubate for 5 minutes at room temperature. - Combine the diluted DNA (with PlurMaPlus™) and PurMaFectin™ Mix from previous step (total volume = 50 µl). - Gently mix. and incubate for 20 minutes at room temperature. - Add 50 µl of complexes to each and gently mix by rocking the plate back and forth. - Change the Medium changed after 4-6 hours with complete media including serum (FBS). - Incubate cells at 37°C in a CO_2 incubator for 18-48 hours.
Day2-3	- Check transfection efficiency 18 to 48 hours post transfection. 48 hours usually gives better results. - Harvest the cells and proceed to the follow up experiments. - The transfection efficiency /cytotoxicity can significantly improve by trying different initial cell density as well as the amount and PurMaFectin™ volume. It is mostly dependent on different expression criteria in the applied mammalian expression vector and the cell type. - During the optimization period, we recommend NOT to use serum and dilute the DNA only with medium.

Table 2.						
		DNA -PurMaPlus™ Complex			PurMaFect™ Mix	
Plate	Well capacity	DNA (µg)	PurMaPlus™(µl)	Medium (µl)	PurMaFect™(µl)	Medium (µl)
96-well	200 µl	0.2	0.4	25	0.5	25
24-well	0.8 ml	1	2	125	2.5	125
6-well	3 ml	2	4	250	5	250
100 mm	10 ml	2	4	250	5	250

Table 3. 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 medium to another. - Perform an optimization for your transfection if you are using a new plasmid, or new cell line and after optimization, DO NOT change the protocol.