PurMa Biologics, LLC

PurMaFectin™ 293T
User Manual

Cat #: P3E212204-01 1X 1ml
Cat #: P3E212204-05 5X 1ml

PurMaFectin™ 293T	
Catalog #	Volume
P3E212204-01	1X 1ml
P3E212204-05	5X 1ml

Protocol		
Time	Description	
Seeding		
Day 0	- Seed the plates Cell 18 to 24 hours before transfection with around 70-80% confluency. - 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™ 293T / DNA complex and the transfection procedures.		
Day1	- Change the media 2-4 hours before transfection. - With PurMaFectin™ 293T, we recommend starting with 1 to 1 ratio. That gives an optimal transfection efficacy with low cytotoxicity. Then if the results are less than 95% successful transfection, increase the ratio of PurMaFectin™ 293T (µl)/DNA (µg) to 2:1. is around 1 to 1. - During the optimization period, we recommend NOT to use serum and dilute the DNA only with medium.	
Procedure		
Day1	- See table 1 for guidelines for culture formats. - The optimal transfection condition may vary in different adherent cell lines. Table 1 is a starting guideline and complete optimization based on the cell line and plasmid criteria needs to be done empirically. - Add the recommended amount of DNA into 150 µl of medium. Vortex and spin down. - For each well/plate/ flask, dilute the recommended volume of PurMaFectin™293T into 150 µl of medium. - Vortex and spin down, incubate at room temperature for 10 minutes. - Add the diluted PurMaFectin™293T dropwise to the diluted DNA solution. - Vortex immediately and spin down. **It is crucial to add the transfection reagent to the DNA mixture NOT in the reverse order.** - Incubate at room temperature for 15 to 20 minutes. - Add the PurMaFectin™293T / DNA mixture drop wise to the medium in each well and homogenize the mixture by gently swirling the plate.	
Day2-3	- Check transfection efficiency 24 to 48 hours post transfection. 48 hours usually gives better results.	

Table 1. PurMaFectin™ 293T Volume and ratio Guide				
Culture Dish/Plate	Media before transfection (ml)	Plasmid (µg)	Medium (µL)	PurMaFectin™ 293T (µL)
12-well	0.5	1-2	1-2	100
6-well	1-2	2-4	2-4	150
6 0 mm	2.5-5	6-12	6-12	250
10 cm	5-10	12-24	12-24	500

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.