Miltenyi Biotec

MACSfectin™ Reagent

0.5 mL	130-098-410
1 mL	130-098-411
5 × 1 mL	130-098-412

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1. Description

Components	**0.5 mL** MACSfectin Reagent (# 130-098-410) or **1 mL** MACSfectin Reagent (# 130-098-411) or **5×1 mL** MACSfectin Reagent (# 130-098-412)
Size	**0.5 mL** for up to 500 transfections in 24-well plates or **1 mL** for up to 1000 transfections in 24-well plates or **5×1 mL** for up to 5000 transfections in 24-well plates
Product format	MACSfectin Reagent is supplied in a sterile solution without preservatives.
Storage	Store at –20 °C. The expiration date is indicated on the vial label.

1.1 Transfection technology

MACSfectin Reagent is based on a novel class of cationic lipopolyamines that optimizes nucleic acid condensation and cellular uptake while augmenting the cytoplasmic release of the nucleic acid cargo. MACSfectin Reagent exhibits high biodegradation characteristics resulting in good transgene expression with low toxicity.

MACSfectin Reagent can be used to transfect a wide range of adherent and suspension cells including mouse ES cells. For a list of selected cell types successfully transfected with MACSfectin Reagent refer to www.miltenyibiotec.com/transfection. Please share your transfection results at transfection@miltenyibiotec.de.

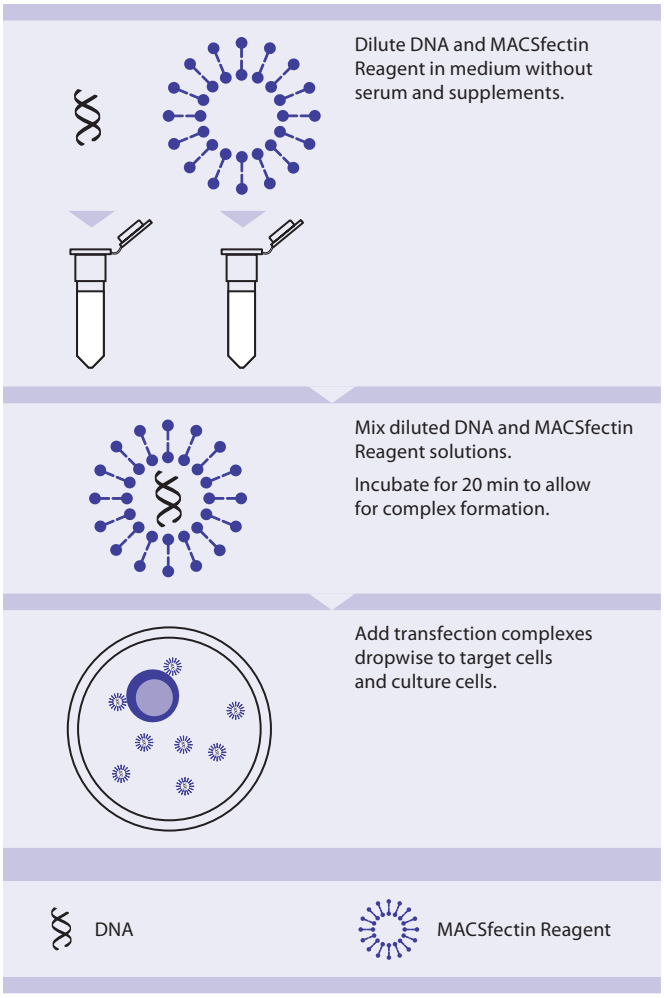


Figure 1: Overview of MACSfectin Reagent transfection protocol.

1.2 Product applications

- Plasmid DNA transfection
- mRNA transfection
- siRNA transfection

1.3 Reagent and instrument requirements

- Cell culture medium without serum and supplements
- Complete cell culture medium including serum and supplements
- Tris-EDTA (TE) buffer: 10 mM Tris-HCl, 1 mM EDTA, pH 8.0
- Cell culture dishes
- 1.5 mL polypropylene or polystyrene tubes
- (Optional) MACS Media

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1.4 Related products

- MACSelect™ 4 – Transfected Cell Selection Kit (# 130-091-988)
- MACSelect K^{k} – Transfected Cell Selection Kit (# 130-091-986)
- MACSelect LNGFR – Transfected Cell Selection Kit (# 130-091-879)
- MACSductin™ Reagent, 0.25 mL (# 130-097-256), 0.5 mL (# 130-097-257), 3×0.5 mL (# 130-097-259)

For all MACSmolecular products and services for molecular analyses: www.macsmolecular.com

2. Protocol for transfection

▲ For every cell type and DNA there is an optimal DNA : MACSfectin Reagent ratio that should be determined experimentally. Examples with a DNA : MACSfectin Reagent ratio of 1:2 for different cell culture formats are shown in tables 2 and 3. It is recommended to use a ratio of 1:2 or 1:3 in initial experiments.

2.1 Cell preparation

2.1.1. For adherent cells

Plate the cells 24 hours before transfection in an appropriate cell culture vessel in complete medium including serum and supplements. For suggested cell numbers see table 1. Cells should generally be >60% confluent on the day of transfection.

2.1.2. For suspension cells

The cells should be cultivated in the exponential growing phase at approximately 2–5×10^{5} cells per mL in complete medium including serum and supplements prior to transfection. On the day of transfection, replate the cells in complete medium including serum and supplements with cell numbers as suggested in table 1.

Cell culture vessel	Adherent cell number (×10^{5})	Suspension cell number (×10^{5})
96 well	0.05–0.2	0.5–1
24 well	0.5–1	1–4
12 well	1–2	2–8
6 well	2–5	5–15
60 mm dish	5–10	10–50
90–100 mm dish	10–30	25–100
T75 flask	20–50	35–130

Table 1: Guidelines for seeding target cells in different cell culture vessels for transfection with MACSfectin Reagent.

2.2 Transfection of adherent cells

Before starting

▲ Thaw MACSfectin Reagent at room temperature and vortex gently.

▲ Warm cell culture medium without serum and supplements to room temperature.

▲ Prepare the transfection complexes directly before the transfection.

▲ The reagent volumes required for different cultivation formats and numbers of adherent cells are listed in table 2.

1. Use high-quality DNA solubilized in sterile water or TE buffer. Calculate the volume of DNA solution required for the transfection. Prepare a polypropylene or polystyrene tube with medium without serum and supplements to dilute the DNA to a final total volume as shown in table 2. Add the desired amount of DNA.

   ▲ **Note:** Do not use serum-containing medium. Serum will inhibit complex formation.

2. Prepare a polypropylene or polystyrene tube with medium without serum and supplements to dilute the MACSfectin Reagent to a final total volume as shown in table 2. Add the desired amount of MACSfectin Reagent.

   ▲ **Note:** Avoid contact of DNA or MACSfectin Reagent with plastic surfaces.

3. Add an equal volume of the diluted DNA solution to the diluted MACSfectin Reagent solution and mix by pipetting up and down 3–5 times. Do not vortex.

4. Incubate the mixture for 20 minutes at room temperature to allow for transfection complex formation.

   ▲ **Note:** Longer incubations are not recommended.

5. Add the complexes dropwise to the cells and mix by gently rocking the plate from side to side to ensure a uniform distribution of the mixture.

6. Incubate the cells under standard conditions for 24–72 hours.

Cell culture vessel	DNA quantity (µg)	Final volume of DNA diluted in medium (µL)	MACSfectin Reagent (µL)	Final volume of diluted MACSfectin Reagent in medium (µL)	Cell culture volume (mL)
96 well	0.25	25	0.5	25	0.1
24 well	1	50	2	50	0.5
12 well	2	50	4	50	1
6 well	3	100	6	100	2
60 mm dish	6	150	12	150	3
100 mm dish	12	250	24	250	8
T75 flask	20	350	40	350	10

Table 2: Examples of MACSfectin Reagent requirements for DNA transfection of adherent cells using a DNA : MACSfectin Reagent ratio of 1:2.

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2.3 Transfection of suspension cells

Before starting

▲ Thaw MACSfectin Reagent at room temperature and vortex gently.

▲ Warm cell culture medium without serum and supplements to room temperature.

▲ Warm cell culture medium including serum and supplements to 37 °C.

▲ Prepare the transfection complexes directly before the transfection.

▲ The reagent volumes required for different cultivation formats and numbers of adherent cells are listed in table 3.

1. Use high-quality DNA solubilized in sterile water or TE buffer. Calculate the volume of DNA solution required for the transfection. Prepare a polypropylene or polystyrene tube with medium without serum and supplements to dilute the DNA to a final total volume as shown in table 3. Add the desired amount of DNA.

   ▲ **Note:** Do not use serum-containing medium. Serum will inhibit complex formation.

2. Prepare a polypropylene or polystyrene tube with medium without serum and supplements to dilute the MACSfectin Reagent to a final total volume as shown in table 3. Add the desired amount of MACSfectin Reagent.

   ▲ **Note:** Avoid contact of DNA or MACSfectin Reagent with plastic surfaces.

3. Add an equal volume of the diluted DNA solution to the diluted MACSfectin Reagent solution and mix by pipetting up and down 3–5 times. Do not vortex.

4. Incubate the mixture for 20 minutes at room temperature to allow for transfection complex formation.

   ▲ **Note:** Longer incubations are not recommended.

5. During the complex formation, prepare cells in pre-warmed medium including serum and supplements at the correct cell density according to table 1. Transfer sample to an appropriate cell culture vessel.

6. Add the complexes dropwise to the cell suspension. To ensure a uniform distribution of the mixture and contact of the complexes with the cells, pipette the culture medium gently up and down 3–4 times.

7. Incubate the cells under standard conditions for 24–72 hours.

Cell culture vessel	DNA quantity (µg)	Final volume of DNA diluted in medium (µL)	MACSfectin Reagent (µL)	Final volume of diluted MACSfectin Reagent in medium (µL)	Cell culture volume (mL)
96 well	0.25	25	0.5	25	0.1
24 well	1	50	2	50	0.5
6 well	3	100	6	100	2
60 mm dish	6	150	12	150	3

Table 3: Examples of MACSfectin Reagent requirements for DNA transfection of suspension cells using a DNA : MACSfectin Reagent ratio of 1:2.

3. Examples: Efficient transfection using MACSfectin Reagent

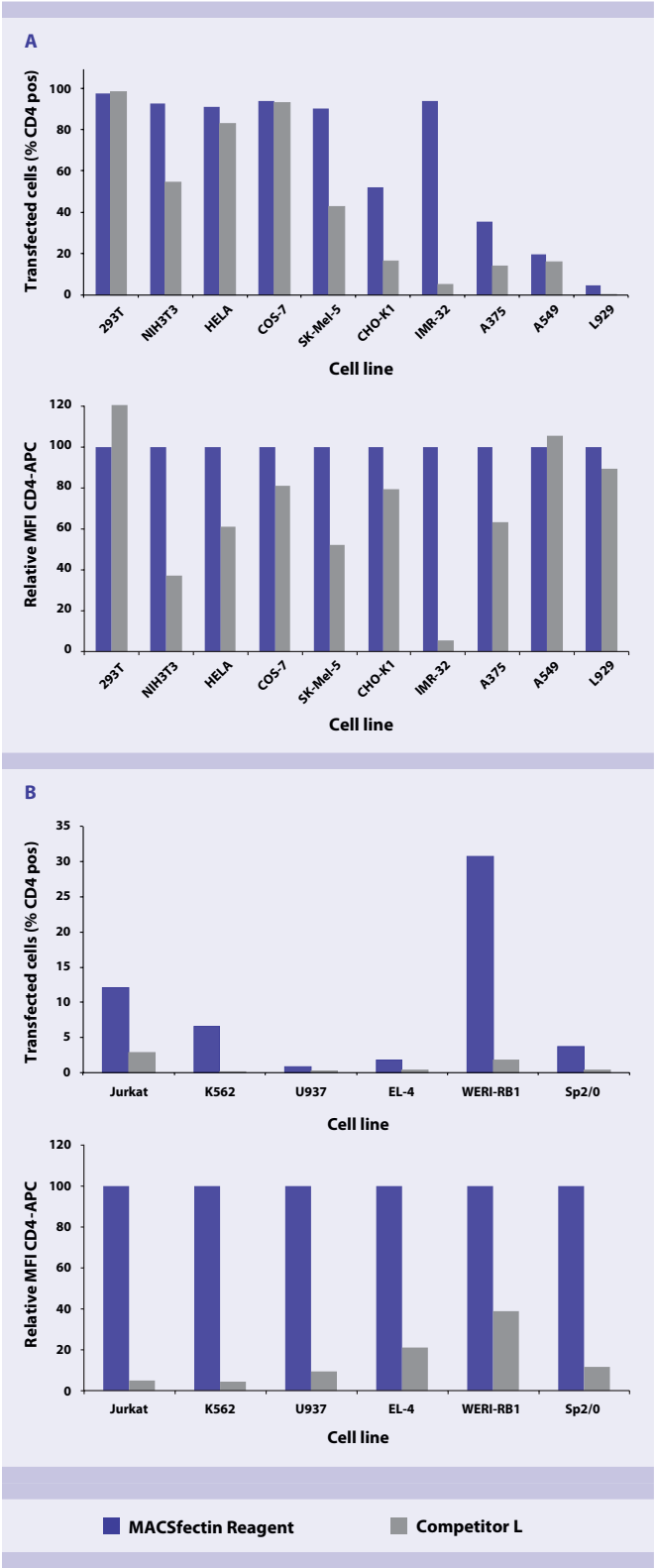


Figure 2: 10 adherent cell lines (A) and 6 suspension cell lines (B) were transfected with a plasmid vector expressing cell surface human CD4 in 6-well plates with MACSfectin™ Reagent and a competitor product using standard transfection conditions (4 µg DNA and 8–16 µL MACSfectin Reagent). The number of transfected cells and mean fluorescence intensity (MFI) were assessed 48 hours post-transfection by staining the cells with CD4-APC and flow cytometric analysis using a MACSQuant® Analyzer. The MFI value of cells transfected with MACSfectin Reagent were set to 100% for comparison with the competitor product.

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4. Troubleshooting

Low transfection efficiency

▲ Suboptimal DNA : MACSfectin Reagent ratio. Optimize the ratio by using a fixed amount of DNA (µg) or siRNA (nM) and vary the amount of MACSfectin Reagent by 3–fold (both increasing and decreasing the ratio) from the recommended values shown in tables 2 and 3.

▲ Suboptimal amount of DNA. Vary the quantity of DNA using the recommended or optimized (see previous note) DNA : MACSfectin Reagent ratio.

▲ Suboptimal cell density. For efficient uptake of transfection complexes, the cells should be in the exponential growth phase. Therefore, adherent cells should be from 60-80% confluent on the day of transfection.

▲ Poor DNA quality. DNA should be endotoxin-free and concentrated to minimize dilution of media.

▲ Suboptimal cell culture conditions. Adherent cells should not be over-confluent or suspension cells grown too dense. High passage numbers can reduce transfection efficiency. Cells should be free of mycoplasma. The correct medium should be used for cell culture.

▲ Medium including serum and supplements used for transfection complex formation. Serum inhibits transfection complex formation and only medium without serum and supplements (or buffers such as PBS) should be used.

▲ Incorrect assay timepoint. The assay timepoint must be optimized for each transgene construct. Promoter activity, transgene half-life and the proliferative capacity of the cells affect gene expression. Generally, rapidly dividing cells can be assayed after 24 hours, slowly dividing cells after 72–96 hours.

▲ Incorrect MACSfectin Reagent storage or handling. MACSfectin Reagent should be stored at –20 °C. Prolonged storage at 4 °C will reduce performance. The reagent can be regenerated by refreezing at –20 °C. The reagent should be warmed to room temperature and gently vortexed before use.

▲ Aggregated transfection complexes. DNA : MACSfectin Reagent complexes should be freshly prepared by incubation at room temperature for 20 minutes. Prolonged incubations >1 hour can lead to aggregation and loss of efficiency.

Reduced cell viability

▲ Suboptimal cell culture conditions. Adherent cells should not be over-confluent or suspension cells grown too dense. Cells should be free of mycoplasma or other contaminants. The correct medium should be used for cell culture. Antibiotics for transgene selection should not be used for the first 24 hours post-transfection.

▲ Toxic transgene or siRNA. Assess transfection conditions by using a control construct and carrying out transfections with and without the transgene.

▲ Poor DNA quality. DNA should be endotoxin-free and concentrated to minimize dilution of media.

▲ Excessive amounts of DNA or MACSfectin Reagent. Lower the amount of DNA used per transfection. Lower the proportion of MACSfectin Reagent used per mass of DNA.

▲ Transfection complex sensitivity. Some cells may be sensitive to prolonged exposure to transfection complexes. Replace the transfection medium 4–16 hours post-transfection with fresh pre-warmed medium. Refer to **www.miltenyibiotec.com** for all data sheets and protocols. Miltenyi Biotec provides technical support worldwide. Visit www.miltenyibiotec.com for local Miltenyi Biotec Technical Support contact information.

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