Applications
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Sample preparation for microinjection

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Abstract

Microinjection is one of the core methods to introduce DNA and other non-permeable molecules into cells. It allows direct access to the two main intracellular compartments, the nucleus and the cytoplasm. Furthermore single cells can be used to study complex cellular processes, structure and function in vivo. However, sample preparation and handling is crucial for achieving successful microinjection and have to be done with great care. This application mainly focuses on microinjection into adherent cells. In addition, useful general information about sample preparation is presented.

Introduction

Microinjection is the loading or transfer of a dissolved substance into a living cell. This is performed with a fine pulled-out capillary. The tip of the glass capillary has an inner diameter between 0.2 µm and 1 µm [1]. This capillary, also called pipette, is filled with the substance to be injected (typical volumes 0.2 to 2 µl). After the capillary has pierced the cell, a certain amount of this solution (typically ~ 10% of the cell volume) is transferred into the cell [2]. As the diameter of the capillary is very small, particles in the injection solution can quickly result in blockage of the capillary. In this case the capillary has to be changed. To optimize the operating lifetime, the sample should be centrifuged for 10-15 minutes at min. 10,000 x g before loading. As the cross-section of the capillary is very small the liquid runs dry very quickly and may clog the capillary. For this reason, a filled capillary should be lowered into the medium immediately after filling and insertion into the holder. To avoid blockage, the capillary should always remain in the medium especially during long experiments when a large number of cells are injected.

The culture medium for microinjection should contain antibiotics to prevent contamination.
Conventional cell culture medium rapidly changes to basic pH when exposed to laboratory atmosphere during microinjection. For some cell types this is often a severe problem. Therefore a culture medium with low bicarbonate concentrations (e.g. 0.85 g/l) buffered with 25 mm HEPES (pH 7.2) should be routinely used. Commercially available CO_2-Independent Medium® (Invitrogen) can also be used. A 3 cm dish should be filled with at least 2 ml medium. After injection further cultivation can take place in the standard cell culture medium.

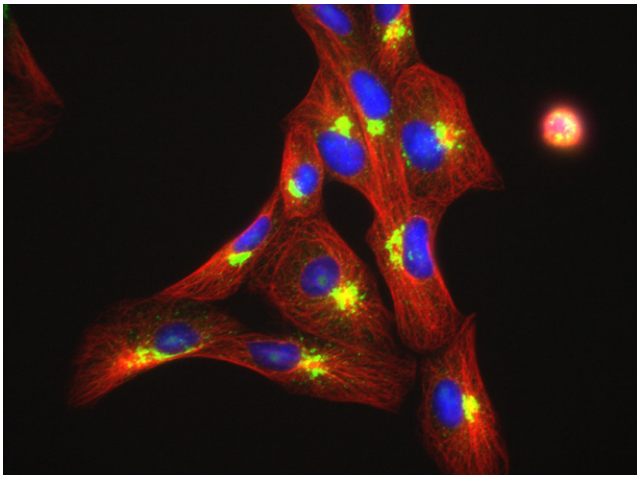

Fig. 1: Adherent cells (labelled with specific antibodies)
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General procedure

1. Plate 250 cells in 5 µl droplets in the center of a glass coverslip (10 x 10 mm).
2. Place coverslips into a humid chamber and incubate at 37 °C until cells attach to the glass (takes usually 6-8 h).
3. Transfer coverslips into 35 mm Petri dishes containing 2 ml of culture medium and let cells grow for at least 24 h before microinjection at 37 °C. The density should be 50% - 70% confluent at time of injection.
4. Microinject cells on the coverslip.
5. Proceed with biochemical analysis (depends on the particular experiment).

Sample Preparation

Proteins and Antibodies

Purification [4] Purification methods which result in highest activity of the particular proteins or antibodies should be used. For peptide antibodies raised in rabbits, affinity purification is recommended. Typical concentrations for proteins and antibodies are 1- 5 mg/ml. The concentration of the material injected should be 10 to 20 times higher than that required for the optimum *in vitro* activity because the sample is diluted 10 to 20 times when injected into the cells.

Storage Purified antibodies must be stored in the concentration in which they arise. Shock freeze small aliquots of 5-10 µl in liquid nitrogen then store at -20°C or even better at -80 °C. Azide should not be used. Refrain from repeated freezing and thawing as antibodies lose activity and lead to blockages of the injection capillaries due to agglutination. Thawing should be performed as quickly and gently as possible.
Before loading the capillary centrifuge material for 15 minutes at 4 °C (10,000 x g). Chill supernatant or load directly into capillary.

Fig. 3: FemtoJet, programmable microinjector with integrated pressure supply

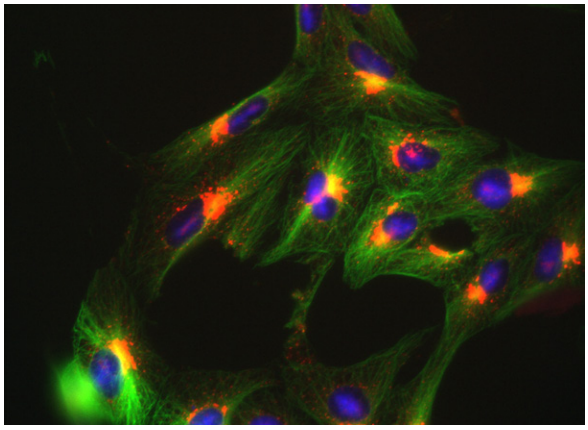

Fig. 2: Adherent cells (labelled with specific antibodies) Dr. Rainer Pepperkok, EMBL Heidelberg

DNA

Purification [5] The highest expression level of microinjected plasmid DNA is achieved when the DNA has been purified by CsCl ethidium bromide gradient centrifugation in accordance with the Maniatis protocol [6]. Today DNA purification kits from different companies also provide DNA of good quality and purity for use in microinjecton. DNA concentrations of 20 ng/µl or up to 200 ng/µl (depending on the activity of the promotor) for plasmid DNA are recommended [1].

Storage Store at -20 °C in small aliquots of 5-10 µl. Thawing should be performed as quickly and gently as possible. Before loading the capillary centrifuge material for 15 minutes at 4° C (10,000 x g). Chill supernatant or load directly into capillary. Repeated centrifugation is not necessary when used within an hour and chilled on ice.

RNA

Purification Any standard protocol is suitable for purifying RNA solutions [6].
Concentrations of 1-2 mg/ml should be used for mRNA and up to 10 mg/ml for total RNA.

Storage Store at -80 °C in small aliquots of 5-10 µl. For longer periods it is advisable to dissolve and store the cleaned RNA in alcohol instead of water.
Thawing should be performed as quickly and gently as possible.
Before loading the capillary centrifuge material for min. 15 minutes at 4 °C (10,000 x g). Chill supernatant or load directly into capillary. Only centrifuge and use each aliquot once.

Dyes

Fluorescent injection markers [1]
Fluorescent injection markers for identification and following of injected cells include: fluorescence labelled dextrans, antibodies or bovine serum albumin. Floures-cent conjugated dextrans can be followed in living cells up to three days after microinjection. As coinjection markers they are used in concentrations of 0.5 - 1 mg/ml.

Storage Store at -80 °C in small aliquots of 5-10 µl. Many solutions are sensitive to light thus exposure to direct light should be avoided. Prepare stock solutions at a concentration of at least 10 mg/ml and filter through a 0.2 µm syringe filter.

Peptides

Please refer to the current literature for information on the cleaning and use of peptides [7].
A concentration of at least 5-10 mg/ml should be used for injection because some peptides rapidly degrade in the cells after injection.

Oligonucleotides

Purification The purification of oligonucleotide solutions is very important. Cleaning with gel or HPLC is recommend-ed. A concentration of 1-2 mg/ml should be used for injec-tion of antisense oligonucleotides with 10-20 bases.

Note:
Injected oligos accumulate easily in the nucleus.

Microinjection buffers

The buffer which is closest to the physiological milieu of the cell contains:
48 mM K_2HPO_4
4.5 mM KH_2PO_4
14 mM NaH_2PO_4, pH 7.2
Whenever possible this buffer should be used as injec-tion buffer. However, several other buffers have been used without any obvious effect on cell function:

Protein
- Phosphate buffered saline (PBS)
- 50 mM HEPES (pH 7.2), 100 mM KCl
- 5 mM $NaPO_4$ (pH 7.3)

Antibody
- 10 mM NaH_2PO_4, 70 mM KCl (pH 7.2)
- 5 mM $NaPO_4$, 50-100 mM KCl (pH 7.2)
- Phosphate buffered saline (PBS)

DNA, RNA, oligonucleotides
- ddH_2O

Note: All buffer solutions should be filtered through 0.2 µm pore size filters before use in order to prevent block-ing of the injection capillaries.

Fig. 2: Workstation for microinjection into adherent cells:
InjectMan NI2 with microscope Zeiss Axiovert 200 and microinjector FemtoJet

Literature

[1] Pepperkok, R., Schneider, C., Philipson, L., and Ansorge, W. (1988). Single cell assay with an automated capillary microinjection system. Exp.Cell Res. 178, 369-376

[2] Pepperkok, R., Scheel, J., Horstmann, H., Hauri, H.P., Griffiths, G., and Kreis,T. E. (1993b). ß-COP is essential for biosynthetic membrane transport from the endplasmic reticulum to the Golgi complex in vivo. Cell 74, 71-82.

[3] Pepperkok, R., Saffrich, R., and Ansorge, W. (1994). Computer-Automated Capillary Microinjection of Macromolecules into Living Cells. Cell Biology: A Laboratory Handbook, CSH Laboratory Press.

[4] Antibodies: A laboratory manual (1988). Harlow, E. and Lane, D. ed., CSH Laboratory Press.

[5] Proctor, G.N. (1992). Microinjection of DNA into mammalian cell in culture: Theory and practise. Methods Mol. Cell. Biol. 3, 209-231.

[6] Sambrook, Fritsch, Maniatis (1989). Molecular Cloning : A laboratory manual. CSH Laboratory Press.

[7] Aguilar, M. (2004). HPLC of Peptides and Proteins: Methods and Protocols (Methods in Molecular Biology). Humana Press Inc.

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Ordering information

Article	Description	Order no. international	Order no. North America
InjectMan®NI2 1)	Dynamic micromanipulator for microinjection	5181 000.017	92000029
FemtoJet 1)	Programmable microinjector with integrated pressure supply	5247 000.013	920010504
Universal Capillary holder		5176 190.002	920007392
Femtotips	20 sterile glass capillaries for microinjection into adherent and suspension cells	5242 952.008	930000035
Femtotips II	20 sterile glass capillaries for microinjection into adherent and suspension cells	5242 957.000	930000043
Microloader	Pipette tip for filling microinjection capillaries, set of 2 x 96 pcs.	5242 956.003	930001007
Pipette Research	0.5 - 10 µl	3111 000.025	022471902
Centrifuge MiniSpin	incl. standard rotor	5452 000.018	022620100

1) Order numbers for country-specific versions of this equipment on request

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