

LNA™ for *in situ* detection of mRNA in fixed cells

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Rat9G cells are grown on microscope slides and immediate early HCMV mRNA expression is induced (in approximately 30% of the cells) by incubating cells with cycloheximide for 4-6 hours. After this:

- Wash cells shortly with PBS
- Fix cells in 4% formaldehyde*/5% acetic acid in PBS for 15 min
- Wash cells 2 x 5 min with PBS
- Treat cells with pepsin (0.1% in 10 mM HCl) for 1 min at 37°C
- Wash cells 2 times shortly with water
- Dehydrate through 70%-, 90%- and 100% ethanol
- Air dry
- Put 10 µL of diluted probe (see below) on a slide and cover with a 18x18 mm cover slip
- Denature probe + slide at 80°C for 75 sec
- Hybridize for 30 min in a humid chamber at hybridization temp = T_m probe -21°C
- Wash off the cover slip by immersion in 2xSSC, 0.1% Tween-20
- Wash 3 x 5 min with 0.1xSSC at 65°C
- Dehydrate through 70%-, 90%- and 100% ethanol
- Air dry
- Bring on 20 µl of DAPI + antifade and cover with a 24x50 mm coverslip

Probe dilution

The LNA™ mRNA detection probe is diluted with H₂O to a 20-40nM concentration in hybridization buffer.

Buffers

Hybridization buffer: 50% deionized formamide, 2xSSC, 50 mM Sodium phosphate pH 7, 10% dextran sulphate

For preparation of buffers please refer to :

Molecular cloning : a laboratory manual / Sambrook, Joseph; Russell, David W. --
3rd ed. -- New York: Cold Spring Harbor Laboratory, 2001.

*Please note: For optimal fixation it may be critical to use fresh formaldehyde solutions. Fresh 4% solutions can be made from 16%, methanol free, formaldehyde or from solid paraformaldehyde (4% w/v).

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