

Basic Parasite Nucleofector® Starter Kit

For parasitic protozoa (not suitable for *Plasmodium falciparum*)

Parasitic Protozoa

There are some 10.000 species of parasitic protozoa displaying different life cycles and infecting various host organisms.

The Basic Parasite Nucleofector® Starter Kit (Cat.No. VMI-1001) should help you to determine the optimal program and Nucleofector® Solution for your parasite-of-interest before using either Basic Parasite Nucleofector® Kit 1 or 2. However, due to the great number of parasitic protozoa transfection results can vary significantly between different species and stages.

The Nucleofector® Technology has been proven to provide high transfection efficiencies in parasitic species such as *Plasmodium berghei*, *Plasmodium yoelii*, *Trypanosoma brucei*, *Toxoplasma gondii*, *Eimeria tenella*, *Leishmania* and *Perkinsus marinus*.

For an updated list of successfully transfected parasites please check our comprehensive celldatabase: www.amaxa.com/celldatabase

Optimization Setup

Solution	Basic Parasite 1		Basic Parasite 2	
	Nucleofector® I	Nucleofector® II	Nucleofector® I	Nucleofector® II
Program 1	U-33	U-033	U-33	U-033
Program 2	X-01	X-001	X-01	X-001
Program 3	D-23	D-023	D-23	D-023

Note !

We recommend including one control sample with cells suspended in Basic Parasite Nucleofector® Solution 1 or 2 together with 5-10 µg DNA, with no program.

Product Description

Cat. No. **VMI-1001 Basic Parasite Nucleofector® Starter Kit**

0.45 ml Basic Parasite Nucleofector® Solution 1
 0.45 ml Basic Parasite Nucleofector® Solution 2
 0.1 ml Supplement P1
 0.1 ml Supplement P2
 10 certified cuvettes
 10 plastic pipettes

Size 10 reactions

Storage and stability Store Nucleofector® Solution and Supplement at 4°C.
 The expiry date is printed on the Solution Box.

Once the Nucleofector® Supplement is added to the Nucleofector® Solution it is stable for three months at 4°C.

Required Material

Note !

Please make sure that the entire supplement is added to the Nucleofector® Solution!

- › Supplemented Nucleofector® Solution at room temperature
- › Supplied certified cuvettes
- › Supplied plastic pipettes
- › Prepared culture dish or reaction tube
- › Prewarm appropriate volume of culture media to 37°C
- › Substrate of interest, highly purified, preferably by using endotoxin free Kits; A260:A280 ratio should be at least 1.8
- › Nucleofector® device
- › Appropriate number of cells (1×10^6 - 5×10^7 cells per sample)

1. Pre Nucleofection®

Note !

Collection and preparation of parasitic Protozoa may differ significantly between species and stages. Please check the respective literature for preparation of different parasitic protozoa before transfection.

For transfection of the blood stages of *P. berghei* (shizont stage) we recommend following the comprehensive preparation and transfection protocol published by Janse *et al.*² Further protocols have been described for *P. yoelii* blood stage shizonts⁴ and bloodstream or procyclic form of *T. brucei*.⁵

2. Nucleofection®

- 2.1** Please make sure that the entire supplement is added to the Nucleofector® Solution!
- 2.2** Prepare appropriate number of culture dishes or reaction tubes by filling with appropriate culture medium and incubate/equilibrate in a humidified 37°C/5% CO₂ incubator before collecting cells
- 2.3** Count the cells
- 2.4** Centrifuge the required numbers of cells (**1×10^6 - 5×10^7 per sample**) at RT and discard as much supernatant as possible

Note !

Centrifugation speed is dependent on parasitic species and stage
Recommended centrifugation speed:

- **16000xg for 5 seconds** (for shizonts of *P. berghei* or *P. yoelii*)
- **1300xg for 10 minutes** (for bloodstream and procyclic forms of *T. brucei*) (centrifugation speed may differ for species other than *P. berghei*, *P. yoelii* and *T. brucei*)

- 2.5** Resuspend the cell pellet carefully in 100 µl room temperature Nucleofector® Solution per sample
- 2.6** Combine 100 µl of cell suspension with 5-10 µg DNA
- 2.7** Transfer cell/DNA suspension into certified cuvette (sample must cover the bottom of the cuvette without air bubbles)

One Nucleofection® sample contains

- › 1 x 10⁶ - 5 x 10⁷ cells
- › 5-10 µg plasmid DNA (in 1-5 µl H₂O or TE)
- › 100 µl Basic Parasite Nucleofector® Solution 1 or 2

- 2.8** Select the appropriate Nucleofector® program
U-033, X-001 or D-023
- 2.9** Insert the cuvette with cell/DNA suspension into the Nucleofector® cuvette holder and apply the selected program
- 2.10** Take the cuvette out of the holder once the program is finished
- 2.11** Add ~500 µl of the equilibrated culture media to the cuvette and gently transfer the sample into culture dish or reaction tube. Use the supplied pipettes and avoid repeated aspiration of the sample. For injection of transfected cells into small laboratory animals less culture medium (e.g. 50 µl) may be added.

3. Post Nucleofection®

Post Nucleofection® processing of cells depends very much on the parasite, the stage and the subsequent application. Experimental procedures may vary from analysis of transiently transfected parasites cultured *in vitro* to drug-selection and analysis of stably transfected parasites in small animal models.

Janse *et al.* describes selection, collection and analysis of transfected blood stages of the rodent malaria parasite *P. berghei* after injection into mice.² Jongco *et al.* shows a further selection approach for the rodent parasite *P. yoelii*.⁴ Analysis of parasites cultured *in vitro* both after transient and stable Nucleofection® have been recently reported for *T. brucei* and *P. marinus*.^{5,6}

Additional Information

Recommended literature on Nucleofection® of parasitic protozoa:

› ***Plasmodium berghei***

1. Janse *et al.* (2006) Mol Biochem Parasitol 145: 60-70
2. Janse *et al.* (2006) Nat Protocols 1(1): 346-356
3. Janse *et al.* (2006) Nat Protocols 1(2): 614-623

› ***Plasmodium yoelii***

4. Jongco *et al.* (2006) Mol Biochem Parasitol 146: 242-250

› ***Trypanosoma brucei***

5. Burkard *et al.* (2007) Mol Biochem Parasitol 153: 220-223

› ***Perkinsus marinus***

6. Fernández-Robledo *et al.* (2007) Mol Biochem Parasitol 157: 44-53

For an updated list of Nucleofector® citations on parasitic protozoa please check our citation database: www.amaxa.com/citations

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