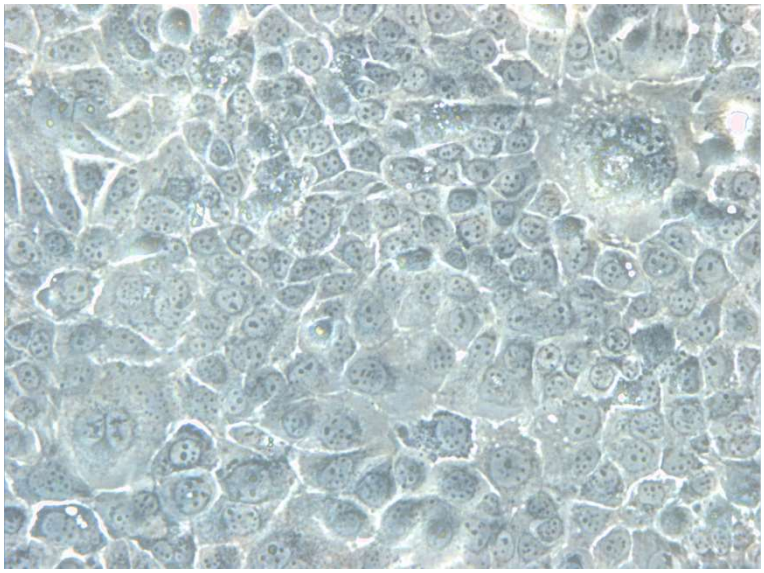
WE SUPPORT YOUR RESEARCH

COLO-680N



Esophageal squamous cell carcinoma

- "The first easy-to-handle system that enables the long-term sustainable production of infective oocysts at a laboratory scale and removes the constant dependence on immunosuppressed animals for production of *Cryptosporidium* oocysts along with all its ethical implications[1]."
- "*C. parvum*-infected cell cultures can be frozen and stored[1]."
- "Unprecedented opportunities to decipher *Cryptosporidium* biology and to develop anti-*Cryptosporidium* therapies[1]."

Contents

COLO-680N esophageal squamous cell carcinoma 3
Product description 3
Applications 3
Media and supplements 3
Cell culture medium 4
Preparing the media for cultivation 4
Getting the cells started after delivery 4
Culture routine 5
Quality controls 5
References 5

COLO-680N esophageal squamous cell carcinoma

Product description

The cell line COLO-680N was established from an esophageal squamous carcinoma of a 57-year old black woman by passaging the tumor through a nude mouse[2].
COLO-680N was now found to be the first sufficient in-vitro platform for long-term production of infective oocysts of *Cryptosporidium parvum*. Prior to the in-vitro model, oocysts had to be freshly acquired from animals. Another important feature is the possibility to deep-freeze the parvum-infected cell cultures without corrupting the oocysts integrity[1].

Applications

- The esophageal squamous-cell carcinoma COLO-680N expresses the bone morphogenetic protein 6 (BMP-6), being involved in keratinogenesis. As the tumor is de-differentiating, BMP-6 expression levels elevate. Therefore BMP-6 could be of use as a co-indicator for prognosis[2].

- COLO-680N now offers the first in-vitro platform for long-term cultivation of Cryptosporidium parvum. The now enabled sufficient production of oocysts allows infection of subsequent cell cultures[1].

Media and supplements

Product name	Size	Order no.
COLO-680N cells	cryovial (ampoule), deep-frozen	300464
COLO-680N growing	2xT25 cell culture flasks	330464
RPMI 1640a	Basic, with glutamine, 500ml. Needs supplementation	820700a
RPMI 1640, ready-to-use	Ready-to-use, with 10% FBS, 500ml. Shelf life of 6-8 weeks, when stored refrigerated. For immediate use or as a starter medium.	820700
CM-1, 50 ml	Cryoprotective medium, with serum, Animal-Component free for mammalian cells; contains 5% DMSO	800150
FBS, EU	Fetal Bovine serum, 50ml, EU-compliant	840903
FBS, US	Fetal Bovine serum, 50ml, US-compliant	840904
PBS	Phosphate-buffered saline, 500ml	850015
Please ask for availability ahead of starting the cell culture.		

Cell culture medium

For the desired proliferation of COLO-680N cells, RPMI 1640 basic (old designation: MG-70a) medium must be supplemented with 10% fetal bovine serum. The Ready-to-use medium (old designation: MG-70), supplemented with 10% FBS, is also available at CLS GmbH.

Preparing the media for cultivation

The RPMI 1640 basic medium comes at room temperature and should be stored at +4°C.
The RPMI 1640 ready-to-use comes cooled at 2 to 8°C, it should be refrigerated.
Serum aliquots must be stored frozen at -20°C until supplementation of the medium.

Getting the cells started after delivery

Delivered deep-frozen on dry ice
If immediate culturing is not intended, the cryovial(s) should be stored below -150°C.

If immediate culturing is intended, please follow these instructions:

- Quickly thaw by rapid agitation in a 37°C water bath within 40-60 seconds. The water bath should have clean water containing an antimicrobial agent.
- As soon as the sample has thawed, remove the cryovial from the water bath (a small ice clump should remain and the cryovial should still be cold).
- From now on, all operations should be carried out under aseptic conditions.
- Immediately transfer the cryovial to a sterile flow cabinet and wipe with 70% alcohol.
- Carefully open the vial and transfer the cell suspension into a 15 ml centrifuge tube containing 8 ml of culture medium.
- Resuspend the cells carefully.
- The cells should be spun down at 300xg for 3 minutes.
- After centrifuging, aseptically remove the supernatant and add 10 ml of fresh cell culture media.
- Carefully resuspend the cells and distribute into one 25cm^2 cell culture flask. Incubate at 37°C/5% CO_2.
- Subculture as soon as the cell concentration has reached 2 x 10^6 cells/ml.
- Within the first 1-3 days of culture the cells may appear not vital, and the viability falls tremendously. The cells will recover and start proliferating (see the graph on page 4).
- It is recommended to distribute the cells into new flasks containing fresh medium instead of adding fresh medium thus diminishing the amount of dead cells and cell debris.

Culture routine

Keep the COLO-680N cells plated at a concentration of 2 to 3 x 10^4 cells/cm^2.
When 80% confluency is reached, aspirate the media supernatant, rinse with PBS and then add an appropriate amount of Accutase (T25 = 2 ml; T75 = 5 ml; T150 = 10 ml) for detachment. Incubate at ambient temperature for 10 minutes. Carefully resuspend the cells, the addition of medium is optional but not necessary, and dispense into new flasks which contain fresh medium. Incubate at 37°C / 5% CO_2.

Quality controls

Cell lines were authenticated by STR profiling.
Absence of SMRV was tested by Real-Time PCR.
Mycoplasma specific PCR: negative; Mycoplasma specific cell-based assay: negative
Bacteria specific PCR: negative

References

[1] Miller CN, Josse L, Brown, IR, Blakeman B, Povey J, Yiangou L, Price M, Cinatl Jindrich, Xue WF, Michaelis M & Tsaousis A. A cell culture platform for *Cryptosporidium* that enables long-term cultivation and new tools for the systematic investigation of its biology. Int. J. Parasitol., 2017. https://doi.org/10.1016/j.ijpara.2017.10.001

[2] Raida M, Sarbia M, Clement JH, Adam S, Gabbert HE, Höffken K. Expression, regulation and clinical significance of bone morphogenetic protein 6 in esophageal squamous-cell carcinoma. Int J Cancer. 83:38–4, 1999.