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FreshCells™ Flask Cultures

For use in the propagation of the cell line for the production of tubes, shell vials, or multi-well plates.

FOR *IN VITRO* DIAGNOSTIC USE

INTENDED USE

FreshCells Flask Cultures are intended to be used for the propagation of the cell line for the production of tubes, shell-vials, or multi-well plates used in patient sample testing and/or research **or** to propagate virus for use as quality control samples associated with patient sample testing or in research. These cells should not be used for serial cell propagation.

SUMMARY

Cultured cells provide the necessary living host systems for the propagation of viruses. The viral propagation procedure typically involves incubating a known virus strain with an appropriately permissive cell line.[1,2] This incubation period is variable and dependent upon the virus. The classic detection method for viral infection in cell culture is the observation of cellular changes due to the infection and replication of the virus, termed cytopathic effect (CPE).

The cell propagation procedure involves passage of the flask cells into multiple tubes, shell-vials, or multi-well plates under appropriate conditions, such that, normal cell growth replenishes the monolayer to the confluence required for viral culture. The incubation period is variable and dependent upon the cell line used.

MATERIALS PROVIDED

- FreshCells flask cultures are provided as cell monolayers adhered to the bottoms of polystyrene flasks (75 cm^2 and 150 cm^2).
- The flasks are filled with medium to reduce shipping stress. Available cell types are found in a Quidel Product Catalog, or by contacting a Quidel Customer Service or Sales Representative.
- Quidel ships the FreshCells flask cultures with a minimum of two (2) passages remaining before the indicated passage limit is reached.

WARNINGS AND PRECAUTIONS

- For *in vitro* diagnostic use.
- As with all methods for virus identification using cultured cells, personnel must be properly trained in virus culture and safe handling techniques[3,4], i.e., manipulations which present potential personnel hazards should be conducted in a Class II biosafety cabinet; and gloves should be worn at all times.
- Cultured cells may also support the replication of infectious agents which are classified by the CDC as agents requiring cultivation under BSL-3 conditions.[5] Consult CDC for listing of the BSL-3 infectious agents and recommendations.

- Cultures should be autoclaved or disinfected with a solution of sodium hypochlorite (1:10 final dilution of household bleach) prior to disposal.
- Testing should be performed in an area with adequate ventilation.
- Dispose of containers and unused contents in accordance with Federal, State and Local regulatory requirements.
- Wear suitable protective clothing, gloves, and eye/face protection when handling the contents of this kit.
- Wash hands thoroughly after handling.
- For additional information on hazard symbols, safety, handling and disposal of the components within this kit, please refer to the Safety Data Sheet (SDS) located at quidel.com.

STORAGE

As with any cell culture, FreshCells Flask Cultures must be protected from extremes in temperature and light. Upon receipt, the cell cultures should be stored at 22°C to 28°C in a clean and dark or low light intensity area and in such a fashion that the monolayers are covered with medium.

If the cells are to be incubated, excess culture medium should be removed, leaving 0.5 to 1.5 cm depth of liquid covering the cells. Incubation conditions are 35°C to 37°C, 5% CO_2 (± 1.0% recommended), 85% to 98% relative humidity to prevent drying.

STABILITY

Upon receipt, FreshCells culture media should be visually examined for a lack of turbidity and the typical peach to pink color. Do not use any media exhibiting a yellow or purple color or any degree of turbidity as these are all indicators of possible contamination.

QUALITY ASSURANCE

FreshCells flask cells are from reliable, reputable, and trackable sources. Prior to acceptance into Quidel production facility, the cell type is reviewed through documentation history and laboratory analysis to verify that no microorganisms (by sterility and *Mycoplasma* testing) and no viruses [as evidenced by the absence of cytopathic effect (CPE)] are found to be present.

NOTE: Concerning cell lines of human origin: Quidel stock inventories of the cell lines of human origin have been tested to verify the absence of HIV and HBV viral DNA using PCR techniques.

Lot Specifications

Prior to shipment of each FreshCells lot*,* representatives of the lot are:

- Screened for the absence of *Mycoplasma spp*. and other adventitious microorganisms
- Planted and the resulting monolayers examined microscopically for morphology, confluence, and uniformity
- Characterized as to species identity by isoenzyme analysis

Information beyond that provided by the Product Insert, Lot Specification Sheet, or Material Safety Data Sheet is available upon request.

PROCEDURE

Preliminary Comments

Cultured cells provide the necessary living host systems for the isolation of viruses. Viral isolation procedures typically involve incubating a prepared clinical specimen with an appropriately permissive cell line (Table 1).

This incubation period is variable and dependent upon the virus.

Table 1. FreshCells *Singles* and Their Virus Susceptibility Profiles

REF	Cell Line/Origin	Infectious Agents
56-	A549 human lung carcinoma	adenovirus, HSV, influenza, measles, mumps, parainfluenza, poliovirus, RSV, rotavirus, VZV, metapneumovirus (MPV)
46-	AGMK African green monkey kidney	influenza, parainfluenza, enteroviruses
53-	BGMK Buffalo green monkey kidney	*Chlamydia*, HSV, coxsackie B, poliovirus
43-	HEL (not CE marked) human embryonic lung	adenovirus, CMV, echovirus, HSV, poliovirus, rhinovirus, vesicular stomatitis (Indiana Strain) virus and VZV
57-	HEp-2 human epidermoid carcinoma	adenovirus, coxsackie B, HSV, measles, parainfluenza, poliovirus, RSV
44-	MRHF (HFF) human foreskin fibroblast	adenovirus, CMV, echovirus, HSV, mumps, poliovirus, rhinovirus, VZV
86-	LLC-MK2 Original Rhesus monkey kidney	poliovirus type 1, enterovirus, rhinovirus, myxovirus and poxvirus groups
58-	Mv1Lu mink lung	HSV, CMV, influenza A, influenza B
54-	McCoy mouse fibroblast	*Chlamydia*, HSV
83-	MDCK Madin-Darby canine kidney	influenza A, influenza B, some types of adenovirus, reoviruses, coxsackie virus
62-	MNA mouse neuroblastoma	Rabies
51-	MRC-5 human fetal lung	CMV, HSV, adenovirus, influenza, mumps, echovirus, poliovirus, rhinovirus, RSV, VZV
76-	RD human rhabdomyosarcoma	adenovirus, echovirus, HSV, poliovirus
48-	RK-p1 (not CE marked) New Zealand White Rabbit	HSV, adenovirus
*See note	RMK Rhesus monkey kidney cells	influenza, parainfluenza, enteroviruses
49-	RhMK rhesus monkey kidney cells	influenza, parainfluenza, enteroviruses
84-	Vero African green monkey kidney cells	adenovirus, coxsackie B, HSV, measles, mumps, poliovirus type 3, rotavirus, rubella
67-	Vero 76 African green monkey kidney cells	adenovirus, coxsackie B, HSV, measles, mumps, poliovirus type 3, rotavirus, rubella, West Nile Virus
63-	CRFK (not CE marked) Cat kidney cortex cells	feline panleukopenia virus, feline calicivirus, reovirus (feline), felid herpesvirus 1, canine parvovirus, rabies virus, bovine viral diarrhea virus 1

* **NOTE**: 16-308-75 (**without** SV5 & SV40 antisera) and 16-309-75 (with SV5 & SV40 antisera) are primary RMK prepared using former ViroMed process; not CE marked.

Cell Propagation

- Propagation of the cells should be performed using established laboratory procedures.
- A representative procedure can be found in most standard virology references.
- In general, cells may be harvested by removing the medium, rinsing the monolayer with HBSS (*without* Ca^{++}or Mg^{++}), adding porcine trypsin at about 2.5 mg/mL at a flask size-to-trypsin volume ratio of approximately 25:1, allowing the trypsin to dissociate the monolayer, then re-suspend the cells in fresh culture medium and distribute among fresh culture vessels.

Virus Propagation

- Propagation of virus should be performed using established laboratory procedures.
- A representative procedure can be found in most standard virology references.
- In general, aseptically remove cell shipping medium from flask, replace with the appropriate cell maintenance medium and inoculate with the virus to be propagated. Incubate under appropriate conditions and examine cell layer until cytopathic effect is at desired levels.

RESULTS

Refer to appropriate reference material for expected results.

QUALITY CONTROL

Uninoculated cell controls should be run with each batch of final cell product to serve as negative controls. Negative controls are handled the same as inoculated monolayers.

Positive virus controls may be run using previously identified viral agents that will produce the desired result (i.e., CPE).

LIMITATIONS

Harsh conditions encountered during shipping may affect the appearance of cell cultures upon receipt. For this reason, all cultures should be examined for appearance and morphology prior to use. Monolayers exhibiting signs of shipping stress (excessive cell rounding or gaps in the monolayer) should be incubated at 35°C to 37°C for up to 24 hours to allow for monolayer recovery. Please contact Quidel Technical Support for further instructions if the monolayers do not appear to recover from the shipping stress. Aging of cell cultures due to passage can result in lower cell yields and the loss of sensitivity to virus replication.

ASSISTANCE

To place an order or for technical support, please contact a Quidel Representative at 800.874.1517 (in the U.S.) or 858.552.1100 (outside the U.S.), Monday through Friday, from 8:00 a.m. to 5:00 p.m., Eastern Time. Orders may also be placed by fax at (740) 592-9820. For e-mail support contact customerservice@quidel.com or technicalsupport@quidel.com.

For services outside the U.S.A., please contact your local distributor. Additional information about Quidel, our products, and our distributors can be found on our website quidel.com.

REFERENCES

1. Viral Culture; Approved Guideline M41-A. Vol. 26, No. 35. Clinical and Laboratory Standards Institute, Wayne, PA. 2006.
2. McAteer J.A., W.H.J. Douglas. Monolayer culture techniques in: W.B. Jakoby (ed): Cell culture Methods in Enzymology, 1979, Vol. 58, p 132-140, Academic Press.

3. Biosafety in Microbiological and Biomedical Laboratories (BMBL), 5th edition, 2009, CDC-NIH manual. [http://www.cdc.gov/biosafety/publications/bmbl5/index.htm]
4. [1]Biosafety Manual, 3rd edition, 2004. World Health Organization [Manual is available in additional languages; refer to WHO web page [http://www.who.int/csr/resources/publications/biosafety/WHO_CDS_CSR_LYO_2004_11/en/]
5. Laboratory Biosafety Guidelines, 3rd edition, 2004. Published by authority of the Minister of Health, Population and Public Health Branch, Centre for Emergency Preparedness and Response [Guideline is available in French or English; refer to web page [http://www.phac-aspc.gc.ca/publicat/lbg-ldmbl-04/index-eng.html]

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REF Catalogue number	CE CE mark of conformity
EC REP Authorized Representative in the European Community	LOT Batch code
 Use by	 Manufacturer
 Temperature limitation	 Intended use
 Consult e-labeling instructions for use	 Biological risks
IVD For *In Vitro* diagnostic use	CONT Contents/Contains