



FreshCells™

Primary Kidney Tissue Culture

For use in the cultivation and amplification of viruses from patient specimens.

FOR *IN VITRO* DIAGNOSTIC USE



INTENDED USE

Primary and 1st passage (p1) kidney tissue cultures are intended to be used in the cultivation and amplification of viruses, such as respiratory viruses, enteroviruses, measles, and poliovirus from patient specimens.

These cells are not to be propagated beyond their intended single use.

Primary monkey kidney (pMK), 1st passage monkey kidney (RhMK II), and rabbit kidney (RK-p1) tissue cultures are used to amplify viruses to a detectable level from patient specimens that are sent by physicians for the purpose of identifying a stated virus or virus group. Patient specimens may have been taken from various body sites, e.g., genital, oral, bronchial-alveolar, CSF, blood, urine, etc.

SUMMARY

Cells used for culturing viruses have been derived from a variety of animal tissues. It is widely accepted that cell cultures are most permissive to viruses when they have been cultured directly from animal tissue. A culture is “primary” when it has been grown directly from a tissue or organ without being passaged.

Simian tissue has been shown to be very permissive to human viruses. PMK cells have been used in virology since the 1950's, when they were found to support the growth of polioviruses.¹ PMK cells are derived from adult animals by enzyme treatment of kidneys; RK-p1 cells are derived from young New Zealand White Rabbits by enzyme treatment of kidneys. The cells are centrifuged free of the enzyme preparation, re-suspended in growth medium, and cultured in appropriate containers.

The classic detection method of viral infection is the observation of cellular changes due to infection of the cells, termed cytopathic effect (CPE). CPE alone is **not** diagnostic and requires additional testing. The use of monoclonal antibodies against specific infectious agents to confirm an agent's identity has become widely accepted; this method has increased the sensitivity of the cell culture system and decreased the time to infectious agent detection.

DESCRIPTION OF THE DEVICE

Cells isolated from the kidneys of *Macaca mulatta* (Rhesus), New Zealand White Rabbit, and *Felis catus* (domestic cat) are cultured in EMEM supplemented with HEPES and FBS in round-bottom tubes, shell-vials and other appropriate containers. It is well recognized that various simian viruses (SV) are endogenous in PMK cultures and can sometimes interfere with the culture of virus contained in the inoculum. Addition of antisera to SV5 and SV40 to the

medium may inhibit the growth of these Simian Viruses and is, therefore, available as an option for the Rhesus products.

PMK and RK-p1 cells are provided as monolayers of islands or cords of flat epithelial cells surrounded by other fibroblast-like cells adhered to the bottoms of glass culture tubes or to glass coverslips on the bottoms of shell-vials. Each monolayer is covered with a volume of antibiotic-containing medium, sufficient to maintain cell viability.

Table 1. Cell Culture Types

Select a “cell source/type”, then a “culture format”.				
Cell Source	Cell Types	Catalog No.	Culture Format	Part Number
Rhesus (<i>Macaca mulatta</i>) (not CE marked)	pRMK	_-309-_	With SV5 & SV40 antisera	§Prefix: 14-, 15-, 16-, 18-
Rhesus (<i>Macaca mulatta</i>) (not CE marked)	pRMK	_-308-_	Without SV5 & SV40 antisera	§Prefix: 14-, 15-, 16-
Rhesus (<i>Macaca mulatta</i>)	pRhMK	49-xxxxA	With SV5 & SV40 antisera	¥49-0600A, -0102A, -24xxA
Rhesus (<i>Macaca mulatta</i>)	pRhMK	49-xxxx	Without SV5 & SV40 antisera	¥49-0600, -0102, -24xx
Rhesus (<i>Macaca mulatta</i>)	RhMK II*	49-xxxxY	1 st passage (age < 3 years)	¥49-0600Y, -0102Y, -24xxY
New Zealand White Rabbit (not CE marked)	RK-p1	48-xxxxP	1 st passage (age 7-14 days)	¥48-0600P, -0102P, -24xxP, -T075P
Domestic cat (<i>Felis catus</i>) (not CE marked)	CRFK	63-xxxx	(age 12 weeks)	¥63-0600, -2424, -T075

§ 14-(16 mm tube), 15-(shell-vial), 16-xxx-75 (75-cm² flask), 18-xxx-24 (24-well plate with 24 fill)

¥ -0600 (16 mm tube), -0102 (shell-vial with coverslip), -24xx (24-well plate with xx fill), -T075 (75-cm² flask)

* All RhMK II culture formats (49-xxxxY) contain SV5 & SV40 antisera.

WARNINGS AND PRECAUTIONS

- For *in vitro* diagnostic use
- PMK cells, like all primary animal cells, may harbor endogenous viruses^{2,3} such as simian viruses, foamy viruses, adenoviruses⁴, Herpes B virus, etc. as well as bacteria and parasites. It should be noted that Herpes B, though innocuous to monkeys can infect humans and cause death. The most common endogenous viruses are SV5 and SV40. Some of these viruses may be expressed only after the cells have been “stressed” by, e.g., environmental factors, age of the culture, or inoculation with a specimen or fresh medium. Consequently, all results obtained using this product should be reviewed by appropriately trained laboratory personnel, aware of the possibility that observed CPE may be caused by an endogenous virus as well as a virus in the specimen.
- For PMK cells, antisera to SV5 and SV40 is added to the cultures at the time of dispensing into final product. Antisera is added to neutralize (if present) SV5/40 viruses. Once neutralized, there is no need to add additional antisera upon refeeding. Antisera **does not** prevent or neutralize foamy virus.

- To aid in determining whether an endogenous virus might be present, negative controls (both with fresh medium and as never-opened cultures) should be used and monitored for any cellular changes.
- Shipping medium contains gentamicin, a reproductive toxin. Gentamicin has been associated with significant nephrotoxicity and/or ototoxicity.
- As with all methods for virus identification using cultured cells, personnel must be properly trained in virus culture and safe handling techniques as described in the CDC-NIH manual³, *Biosafety in Microbiological and Biomedical Laboratories*, 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 used for virus identification may also support the replication of infectious agents which are classified by the CDC as agents requiring cultivation under BSL-3 conditions. Consult CDC listing of the BSL-3 infectious agents and the CDC recommendations.
- Cultures and specimens should be autoclaved or disinfected with a solution of sodium hypochlorite (1:10 final dilution of household bleach) prior to disposal
- It is strongly recommended that cultured cells are inoculated within 7 to 9 days of receipt.
- 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 must be protected from extremes in temperature and light.

Upon receipt, cell cultures should be screened for confluency. During shipment some retraction of the cells may occur. Monolayers less than 60% confluent may need to be placed in a 35°C to 37°C incubator overnight and reassessed the next day.

Once the desired confluency is reached, cell monolayers may be inoculated at that time.

If the cells are not to be inoculated, the recommended storage is 22°C to 28°C in a clean, dark or low light intensity area and in such a fashion that the monolayers remain covered by cell culture medium. This action will not affect cell function or viability.

If desired, uninoculated cells can be stored at 35°C to 37°C in an incubator. Incubated conditions **may not be ideal for all primary kidney cell lines** due to the potential of overgrowth, which may result in piling or peeling of the monolayers.

STABILITY

Cell monolayers exhibiting patches of dead or toxic cells (often these cells are refractile) are unacceptable for culture and must be discarded. Uninoculated monolayers containing atypical cells or cytopathic effect (specific cellular change associated with viral infection) also should be discarded.

The medium bathing the uninoculated monolayers should be amber to orange in color and be clear. Tubes or shell-vials containing bacterial (turbid) or mold contamination or exhibiting a shift in pH to basic (magenta or purple) or acidic (yellow), should be discarded.

QUALITY ASSURANCE

Negative controls should be run with each batch of specimens tested for virus or *Chlamydia*. Negative controls consist of uninoculated monolayers that otherwise are handled the same as inoculated monolayers.

Positive virus controls may be run using previously identified viral agents that will produce the result desired from a positive patient sample. While generally not required by regulatory organizations, these may be useful for troubleshooting purposes or for the production of additional external staining controls

PROCEDURE

Preliminary Comments and Precautions

- Use a fresh, sterile pipette for each specimen to avoid cross contamination.
- When inoculating a specimen into a culture, be careful to not splash the residual liquid from the pipette, since it could contaminate adjacent cultures.
- Cells must not be allowed to dry at any stage during the cultivation process.
- To optimize virus cultivation characteristics, the cells to be used for culture amplification must be incubated at 35°C to 37°C for at least 2 hours prior to specimen inoculation. To minimize overgrowth before inoculation of specimens, the cells should be used within 24 hours after placing them in the incubator.
- Warm refeed medium to 25°C to 37°C before adding to cultures.

Suggested Tube Protocol

1. Prior to inoculation, examine the monolayers for proper morphology. Incubate cell cultures for 2- to 16 hours at 35°C to 37°C.
NOTE: This step should be considered separately from any prior incubation to improve monolayer confluency.
2. Remove the shipping medium by gentle aspiration being careful not to touch the aspirator tip to the monolayer. Be sure to remove all of the shipping medium. This is important for respiratory virus cultures as the shipping media contains FBS which can be potentially inhibitory to influenza virus isolation.
3. Add 1.0 to 2.0 mL of the appropriate Refeed Medium to each tube.
4. Transfer 0.2 to 0.4 mL of clinical specimen to each tube.
5. Incubate at 35°C to 37°C in a tube rotation drum at 1 to 3 rpm or in a stationary rack as appropriate for the agent suspected.
6. Read for characteristic cytopathic effect (CPE), and/or
7. Fix and stain according to Quidel protocol or, alternatively, the diagnostic manufacturer's monoclonal antibody staining protocol.
8. Examine the monolayer for fluorescent cells using a fluorescence microscope according to Quidel protocol or, alternatively, the diagnostic staining reagent manufacturer's protocol.

Suggested Shell-vial Protocol and 24-well Plate Protocol

1. Prior to inoculation, examine the monolayers for proper morphology. Incubate cell cultures for 2 to 16 hours at 35°C to 37°C.
NOTE: This step should be considered separately from any prior incubation to improve monolayer confluency.
2. Remove the shipping medium by gentle aspiration being careful not to touch the aspirator tip to the monolayer. Be sure to remove all of the shipping medium. This is important for respiratory virus cultures as the shipping media contains FBS which can be potentially inhibitory to influenza virus isolation.
3. Add 0.5 to 1.0 mL of the appropriate Refeed Medium to each shell-vial.
4. Transfer 0.2 to 0.4 mL of clinical specimen to each shell-vial.
5. Centrifuge the inoculated cultures at 700 x g for 60 minutes at ambient temperature.

6. Incubate at 35°C to 37°C, multi-well plates require a humidified (85 to 95% to prevent drying) and 5% CO₂ (± 1.0% recommended) environment, for 16 to 24 hours, 40 to 48 hours, or longer as appropriate for the agent suspected, or according to the laboratory's established protocol. Additional incubation may be necessary to produce visible CPE prior to staining.
7. Fix and stain according to Quidel protocol or, alternatively, the diagnostic staining reagent manufacturer's protocol.
8. Remove the coverslip using a bent teasing needle (commonly used to make tease preps in a Mycology Laboratory). Place the coverslip cell-side down over a small drop of mounting medium on a glass slide.
9. Examine the monolayer for fluorescent cells using a fluorescence microscope according to Quidel protocol or, alternatively, the diagnostic staining reagent manufacturer's protocol.

LIMITATIONS

Aging of cell cultures can result in loss of sensitivity to virus production. Harsh conditions encountered during shipping may affect the shelf life of cell cultures. For this reason, all cultures should be examined for appearance and morphology prior to inoculation with the specimen. Cells exhibiting poor appearance or morphology should not be used. Cell monolayers will degrade over time as the cells begin to die. The rate of this degradation will vary based on a number of factors (i.e., toxicity of the specimen, age of the cells, etc.). All cellular changes should be compared to the negative control to determine if the changes are due to viral replication. This is another reason why CPE alone is **not** diagnostic for a specific viral pathogen. Final identification of any infectious agent requires the use of a separate diagnostic reagent.

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.

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REF

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GLOSSARY

REF

Catalogue number



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

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