QUIDEL

D³ The Power of Direct Detection®

D³ Enterovirus Family Kit/Reagents

Frequently Asked Questions

General

Where can I find the Package Insert for the D³ Enterovirus Family Kit/Reagents?
You can find the Package Inserts for all Quidel products at quidel.com.

What detection technology is used for the D³ Enterovirus Family Kit/Reagents?

- The D³ IFA Enterovirus Identification Kit/D³ IFA Enterovirus Reagent Duo Pack uses a blend of Enterovirus VP1 antigen-specific murine monoclonal antibodies (MAbs) that when combined with a fluorescein isothiocyanate labeled anti-mouse antibody allow rapid identification of enteroviral antigens in cell culture.
- The D³ Enterovirus 71 DFA Reagent uses specific Enterovirus 71 murine monoclonal antibodies (MAb) labeled with fluorescein isothiocyanate for rapid identification of Enterovirus 71 antigens in cell culture.
- The D³ Enterovirus 71/Coxsackie A16 DFA Reagent uses a non-specific Enterovirus 71 monoclonal antibody (MAb) that reacts with Coxsackievirus A16, labeled with fluorescein isothiocyanate for rapid identification of Enterovirus 71 and Coxsackievirus A16 antigens in cell culture.

What are the microscope requirements for using the D³ Enterovirus Family Kit/Reagents?
The D³ Enterovirus Family Kit/Reagents require a fluorescent microscope of 200X to 400X magnification with a filter set for fluorescein isothiocyanate (FITC) {490 nm-520 nm}.

Specimen Collection, Storage, and Handling

What sample types can I use with the D³ Enterovirus Family Kit/Reagents?
The recommended specimen types are throat swabs or washes, cerebral spinal fluid (CSF), ocular tissue, vesicular or ulcerative lesion, and stool. Refer to the CLSI Viral Culture Approved Guidelines for additional specimen types.

Can the ESwab™ be used with the D³ Enterovirus Family Kit/Reagents?
No. The ESwab is a bacterial collection swab and should not be used for collection of samples for viral detection.

What types of transport media are acceptable for use with the D³ Enterovirus Family Kit/Reagents?
Any transport media that has been approved to support the growth of virus (i.e. UTM, VCM, M4, M4-RT etc.)

How should specimens be handled and stored prior to processing?
Specimens should be stored at 2°C to 8°C and kept for a maximum of 72 hours. Specimens that will not be processed within this timeframe should be frozen and stored at –70°C or colder until use.

Avoid exposing specimens to freeze-thaw cycles or storage at temperatures warmer than –70°C. Such practices are detrimental to viral viability.

Kit Formats

What kit formats are available and what does each kit include?

01-050000 D³ IFA Enterovirus Identification Kit		
N/A	X1	D³ IFA Enterovirus MAb Reagent (5 mL)
N/A	X1	D³ Anti-mouse Conjugate (5 mL)
01-00081	X5	Enterovirus Antigen Control Slides
01-090025	X1	40X PBS Concentrate (25 mL)
01-002007b	X1	Mounting Fluid (7mL)(pH 8.0-8.4)

01-050001 D³ IFA Enterovirus Duo Pack		
N/A	X1	D³ IFA Enterovirus MAb Reagent (5 mL)
N/A	X1	D³ Anti-Mouse Conjugate (5 mL)

Individual Components

- 01-057005 D³ Enterovirus 71 DFA Reagent (5 mL)*
- 01-058005 D³ Enterovirus 71/Coxsackie A16 DFA Reagent (5 mL)*
- 01-00080 Enterovirus Antigen Control Slides (10 pack)
- 01-380100 Super E-Mix Refeed Medium (100 mL)
- 01-320100 RM-02 Refeed Medium, 2% FBS (100 mL)
- 01-320500 RM-02 Refeed Medium, 2% FBS (500 mL)
- 01-090025 40X PBS Concentrate (25 mL)
- 01-002007bMounting Fluid (7 mL)(pH 8.0 to 8.4)

*These are made-to-order products. For information about lead time please contact Customer Service at 800.874.1517 option 1, 3 or 858.552.1100 (outside the U.S.).

Storage

How should D³ Enterovirus Family Kit/Reagents be stored?

All D³ Enterovirus Family Kit/Reagents should be stored at 2°C to 8°C in the dark. See below for cell storage conditions.

What is the shelf life for D³ Enterovirus Family Kit/Reagents and Refeed Medium?

Kit / Media	**Expiration from date of Manufacture**	**Minimum Shipment Expiration**
D³ IFA Enterovirus Identification Kit	9 months	90 days
D³ IFA Enterovirus Duo Pack	9 months	90 days
D³ Enterovirus 71 DFA Reagent	6 months	90 days
D³ Enterovirus 71/Coxsackie A16 DFA Reagent	6 months	90 days
Super E-Mix Refeed Medium	6 months	90 days
RM-02 Refeed Medium (2% FBS)	4 months	60 days

Cells

FreshCells™

What are Super E-Mix™ cells?
Super E-Mix cells are a mixture of human lung carcinoma (A549) and Buffalo green monkey kidney with Degradation Accelerating Factor (sBGMK).

What are the available formats for Super E-Mix cells?
Super E-Mix Mixed cells are available in FreshCells shell-vials (with or without coverslips), multi-well plates (various fill styles and formats) and ReadyCells**.**

How should Super E-Mix Mixed cells be stored?
Due to the dissimilar growth rates of the cells in the mixed cell lines, Super E-Mix Mixed cells should be stored at room temperature (22°C to 28°C) in the dark.

Can Super E-Mix Mixed cells be stored in an incubator set at 35°C to 37°C?
Due to dissimilar growth rates of the two mixed cell lines, storage at 22°C to 28° C in a clean, dark or low light intensity area may be desirable for optimal cell function.

I received my cells today and they look rounded, exhibit holes throughout the monolayer, and/or are subconfluent. What should I do?
We suggest you incubate these cells overnight at 35°C to 37°C and assess the monolayers the next day. If they have improved, store them in the dark at 22°C to 28°C until ready to use. If cells do not improve after overnight incubation, please call Quidel Technical Support. Prior to inoculation, you should pre-incubate healthy Super E-Mix Mixed cells for 2-16 hours at 35°C to 37°C.

I left my inoculated Super E-Mix cultures in the centrifuge overnight. Can I still use them?
You may incubate these cultures at 35°C to 37°C for the recommended 48-72 hours (for the first time point) and 5-7 days (if using additional time points). Then process them as usual. As long as your controls perform as expected, any positive results may be reported. However, all negative results must be repeated using fresh cells. Alternatively, you may reset the entire run using the original sample.

ReadyCells®

What are Super E-Mix ReadyCells?
Super E-Mix ReadyCells are frozen cultured cell monolayers that expand the utility of cultured cells by providing laboratories greater flexibility. ReadyCells are cryopreserved at optimum confluency and sensitivity. They are supplied to the laboratory ready to thaw, refeed and use.

How are Super E-Mix ReadyCells shipped?
ReadyCells are shipped frozen and completely covered in dry ice on all sides.

Can ReadyCells be re-frozen?
Once thawed, ReadyCells cannot be re-frozen.

Can I store ReadyCells in liquid nitrogen?
Do not store ReadyCells in liquid nitrogen.

What temperature should I store Super E-Mix ReadyCells?
Super E-Mix ReadyCells must be stored frozen at –70°C or lower.

My shell vial is missing the coverslip, what should I do?
ReadyCells are prepared by filling the shell vials containing the coverslip with a cell suspension. The vials are then incubated where the cells settle and attach to the coverslip. They are grown to confluence then flash frozen. If the coverslip is missing, contact Technical Support to investigate.

Super E-Mix Refeed Medium

What refeed media should I use with my Super E-Mix FreshCells or ReadyCells*?*
Super E-Mix Refeed Medium is the recommended media since it was formulated specifically for Super E-Mix cells.

I have run out of Super E-Mix Refeed Medium. Can I substitute with a different media?
Yes. RM-02 Refeed Medium (2% FBS) may also be used for Super E-Mix cells.

I left my Super E-Mix Refeed Medium or RM-02 Refeed Medium out of the refrigerator for more than 24 hours. Can I still use it?
We cannot guarantee the performance quality of the medium if it is not stored under proper conditions. As such, we do not recommend using any media that is not correctly stored.

What sizes are available for the Super E-Mix Refeed Medium and the Standard 2% Refeed Media?
FreshCells Super E-Mix Refeed Medium is available in a 100 mL bottle. RM-02 Refeed Medium (2% FBS) is available in a 100 mL and a 500 mL format.

Fixatives

What do I use as a fixative?
For cell spots and shell vials, 100% fresh chilled acetone. An 80% aqueous acetone solution is needed for multi-well plates.

Can I use 100% acetone on a multi-well plate?
The use of 100% acetone in the polystyrene well may cause it to craze and cloud the plastic, making it difficult to examine the monolayers.

What if my culture fixes longer than 10 minutes?
Fixing for longer than 10 minutes can be problematic in plates, causing crazing. Fix with 80% aqueous acetone solution and for 5-10 minutes. Over-fixing can also affect antigen presentation on slides and shell vials.

Can I use methanol as a fixative?
The D³ IFA Enterovirus Identification Kit was tested using an 80% aqueous acetone solution (multi-well plates) or 100% fresh chilled acetone (shell-vials and slides) as the fixative. Acetone is used during routine QC testing as well as for stability studies. We are unable to guarantee the quality and accuracy of the results if another fixative is used. Using methanol as a fixative could result in inaccurate results.

Quality Control

What is the recommended quality control protocol D³ Enterovirus Family Kit/Reagents?
For Reagents

- A fresh Enterovirus Antigen Control Slide should be stained each time the staining procedure is performed.
- Positive and negative controls must demonstrate appropriate fluorescence for specimen to be considered valid.

For Cell Culture

- Negative controls should be run with each batch of specimens tested for virus or chlamydiae.
- Positive controls, though not generally a requirement from regulatory organizations, may be useful for troubleshooting purposes or for the production of additional external staining controls.
- If control cultures fail to perform correctly, results are considered invalid.

Common Inspection Questions

Were the tube monolayer cultures incubated for a sufficient time to recover the viruses for which service is offered?

This only applies to **tube culture** and not to spin amplified shell-vials or multi-well plates. Following the manufacturer's directions should be adequate for spin amplified shell vial protocols. Tube culture recommendations can be found in the CLSI approved guidelines for viral culture.

Were the media and diluents checked for sterility and pH?

The laboratory can follow the guidelines on the Quidel Quality Assurance documents and review cell cultures and media for appropriate color, indicating the proper pH. Before these items leave our facility, they are checked for sterility and pH, and retains are reserved for the life of the lot of the item to ensure that there are no quality changes before the products expire. The Lot Specification Report (LSR is included in the shipment) is indicative that the media and diluents have passed our quality assurance testing. The Quality Assurance documents and LSR are available for immediate download at quidel.com.

Procedural – General

What are the microscope requirements?

Fluorescent microscope with the appropriate filter combination for FITC (excitation peak = 490 nm, emission peak = 520 nm); magnification 200X to 400X.

How many monolayers are suggested for cell culture per specimen?

One monolayer for each screening time point, for example, if screening occurs at 48-72 hours and 5-7 days, a total of two monolayers will be needed.

Can reagents from other manufacturers be used with the D³ Enterovirus Family Kit/Reagents?

Use of other reagents than those specified with the components of this kit may lead to erroneous results.

Does the incubator need to be CO_2 buffered?

For multi-well plates, CO_2 buffering is required. Tubes and shell vials do not require CO_2 buffering.

Do I have to run a positive control?

Positive controls, though not generally a requirement from regulatory organizations, may be useful for troubleshooting purposes or for the production of additional external staining controls.

Will the D³ Enterovirus Family Kit/Reagents detect various sub-types?

In clinical studies the D³ IFA Enterovirus Identification Kit was able to detect known subtypes. See the Specific Performance Characteristics section of the Package Insert.

Does the D³ Enterovirus Family Kit/Reagents subtype?

Kit	Subtype
D³ IFA Enterovirus Identification Kit	**Does not** differentiate between subtypes
D³ IFA Enterovirus DuoPack	**Does not** differentiate between subtypes
D³ Enterovirus 71 DFA Reagent	Enterovirus 71
D³ Enterovirus 71/Coxsackie A16 DFA Reagent	Enterovirus 71/Coxsackie A16 – does not differentiate between the two

There are crystals in my 40X PBS Concentrate is it OK to use?
The **40X PBS** Concentrate may crystallize when stored at 2°C to 8°C. The crystals will dissolve when warmed to ambient temperature.

Can I buy the D³ IFA Enterovirus Reagent or the D³Anti-mouse Conjugate individually?
The D³ IFA Enterovirus MAb Reagent and the D³ Anti-mouse Conjugate are sold as a Duo Pack or in the D³ IFA Enterovirus Identification Kit only.

Procedural – D³ IFA Enterovirus Identification Kit / D³ IFA Enterovirus Duo Pack

Does the D³ IFA Enterovirus Identification Kit / D³ IFA Enterovirus Duo Pack cross-react with any known viruses?
There is limited cross-reactivity with adenovirus types 11 and 16.

Does the D³ IFA Enterovirus Identification Kit / D³ IFA Enterovirus DuoPack detect parechovirus?
No. Human parechoviruses and human enteroviruses share many epidemiologic and clinical characteristics, but they belong to genetically and biologically distinct genera. Echoviruses 22 and 23 were reclassified to the parechovirus genus.

What is the staining pattern of an infected cell?
Cytoplasmic, with bright apple-green fluorescence. Positive cells should exhibit bright apple green fluorescence with a **cytoplasmic** staining pattern.

How should I interpret dull nuclear staining?
Any nuclear non-specific fluorescence present in cultured cells or on Antigen Control Slides should be interpreted as negative for enterovirus. A positive result should be a cytoplasmic bright apple-green fluorescence.

Do I have to use the D³ IFA Enterovirus Reagent before the Anti-Mouse Conjugate?
Yes. Infected cells will not fluoresce if the D³ Anti-mouse Conjugate is used before the D³ IFA Enterovirus MAb Reagent.

Procedural – D³ Enterovirus 71 DFA Reagent and D³ Enterovirus 71/Coxsackie A16 DFA Reagent

Does the D³ Enterovirus 71/Coxsackie A16 DFA Reagent differentiate between the Enterovirus 71 and the Coxsackie A16?
The D³ Enterovirus 71/Coxsackie A16 DFA Reagent does not differentiate between Enterovirus 71 and Coxsackie A16.

References

1. Yung T. Huang, Paul Yam, Huimin Yan, and Yan Sun (2001). Engineered BGMK Cells for Sensitive and Rapid Detection of Enteroviruses. Journal of Clinical Microbiology, Feb. 2002, p. 366-371.
2. George E. Buck, Marise Wiesemann, Linda Stewart (2002). Comparison of mixed cell culture containing genetically engineered BGMK and CaCo-2 cells (Super E-Mix) with RT-PCR and conventional cell culture for the diagnosis of enterovirus meningitis. Journal of Clinical Virology 25 S13-S18.
3. Lynn Yihong Miao, Christina Pierce, Jennifer Gray-Johnson, Jill DeLotell, Carl Shaw, Nate Chapman, Elaine Yeh, David Schnurr, and Yung T. Huang (2009). Monoclonal Antibodies to VP1 Recognize a Broad Range of Enteroviruses. Journal of Clinical Microbiology, Oct. 2009, p. 3108-3113.

The performance of any molecular test is dependent on sample collection and handling and the adherence to the Package Insert.

Refer to the Package Insert on our website at **quidel.com** for additional performance claims.

* For State by state fee schedule, go to www.cms.gov.

**Under federal and state law, it is the individual provider's responsibility to determine appropriate coding, charges and claims for a particular service. Policies regarding coding and payment levels can vary greatly from payer to payer and change over time. Quidel Corp. strongly recommends that providers contact their own regional payers to determine appropriate coding and charge or payment levels prior to submitting claims.