FITC conjugate

Sheep anti-mouse

REF 9 VIR-FITC Σ 200 Tests Product Information File (PIF)

IVD CE 0344 ***In Vitro Diagnostic medical device***

INTERNATIONAL PRODUCT INFORMATION FILE (PIF)

ENGLISH – FRANÇAIS –DEUTSCH – ITALIANO – PORTUGUES - ESPAÑOL

This product is registered as "in vitro diagnostic use" in the countries that belong to the European Community. In all other countries it should be labeled "for research use only".

FITC-conjugated sheep anti-mouse-immunoglobulins

IVD ***In Vitro Diagnostic medical device***

This product is registered as "in-vitro diagnostic use only" as a component of the CMV Brite™ Turbo kit in the countries that belong to the European Community. All obligatory and necessary information as specified in Directive 98/79/EC needed to use this product safely and properly, can be found in the product manual of the CMV Brite™ Turbo kit (please see attachment).

	Description
Host	Sheep
Specificity	All mouse IgG subclasses
Form	$F(ab)_2$ fragments of purified sheep immunoglobulins FITC-conjugated
Applications	Sheep anti-mouse can be used as a second antibody in combination with the C10/C11 cocktail VIR-CMV C10/C11 to detect CMV lower matrix protein pp65
Usage	Ready-to-use solution

Immunofluorescence staining with VIR-CMV C10/C11 and VIR-FITC

1. Prepare cytocentrifuge slides (according to laboratory procedures).
2. During immunofluorescence staining do not allow the cell preparations to dry out.
3. For controlling the immunofluorescence staining you can use control slides (VIR-CMV CS05) produced by IQ Products.
4. Rehydrate control slide in PBS for 1 - 2 minutes.
5. Remove the slide from the washing solution. Carefully dry the area surrounding the cell spot. (Remove one slide at the time to prevent the cells from drying.)
6. Apply 35 µl of C10/C11 (VIR-CMV C10/C11) moab solution and incubate for 20 minutes at 37 °C in a humid chamber.
7. Dip slides 3 times in washing solution PBS for 3 minutes.
8. Remove one slide at a time from the washing solution. Carefully dry the area surrounding the cell spot.
9. Apply 35 µl of FITC-conjugated sheep anti-mouse immunoglobulins with Evans Blue (VIR-FITC).
10. Incubate for 20 minutes at 37 °C in a humid chamber.
11. Wash twice in fresh PBS and carefully rinse with tap water (3 times). Mount with mounting medium and a micro cover glass.
12. Perform reading as soon as possible. Cover the slides tightly in order to minimize fading.

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Handling and Storage

They are supplied in 0.01 M sodium phosphate, 0.15 M NaCl; pH 7.3, 0.2% Evans Blue, 0.09% sodiumazide (NaN_3). Store the vials at 2-8 °C. $F(ab)_2$ fragments should be protected from prolonged exposure to light. Reagents are stable for the period shown on the vial label when stored properly

Warranty Products sold hereunder are warranted only to conform to the quantity and contents stated on the label at the time of delivery to the customer. There are no warranties, expressed or implied, which extend beyond the description on the label of the product. IQ Products is not liable for property damage, personal injury, or economic loss caused by the product

Characterization

To ensure consistently high-quality reagents, each batch of monoclonal antibody is tested for conformance with characteristics of a standard reagent.

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Warning All products contain sodiumazide. This chemical is poisonous and hazardous. Handling should be done by trained staff only

Immunoglobulines de mouton anti-IgG de souris conjuguées au FITC

IVD ***Dispositif médical de diagnostic in vitro***

Ce produit est enregistré comme «strictement réservé au diagnostic in vitro» en tant que composant du Turbo kit CMV Brite™ dans les pays de la Communauté européenne. Tel que spécifié par la Directive 98/79/EC, l'ensemble des informations obligatoires et nécessaires à une utilisation sûre et convenable de ce produit se trouvent dans le manuel du Turbo kit CMV Brite™ (voir l'annexe).

Description

Hôte	Mouton
Spécificité	L'ensemble des sous-catégories d'anti-souris lgG
Forme	Fragments $F(ab)_2$ d'immunoglobulines de mouton conjuguées au FITC purifiées
Applications	Le mouton anti-souris peut être utilisé comme anticorps secondaire en combinaison avec le mélange C10/C11 (VIR-CMV C10/C11) afin de détecter la protéine matricielle pp65 du CMV
Utilisation	Solution prête à l'emploi

Coloration par immunofluorescence avec les combinaisons VIR-CMV C10/C11 et VIR-FITC

1. Préparer les lames de la centrifugeuse (conformément aux procédures de laboratoire).
2. Ne pas laisser les préparations sécher pendant la coloration par immunofluorescence.
3. Pour vérifier la coloration par immunofluorescence, vous pouvez utiliser les lames de contrôle (VIR-CMV CS05) d'IQ Products.
4. Réhydrater la lame de contrôle dans du PBS pendant 1 ou 2 minutes.
5. Retirer la lame de la solution de lavage. Sécher soigneusement la zone entourant la tâche cellulaire. (Retirer une lame à la fois afin d'éviter l'assèchement des cellules).
6. Déposer 35 µl de solution d'anticorps monoclonal C10/C11 (VIR-CMV C10/C11) puis laisser incuber 20 minutes à 37 °C en chambre humide.
7. Tremper les lames trois fois dans la solution de lavage au PBS pendant 3 minutes.
8. Retirer une lame à la fois de la solution de lavage. Sécher soigneusement la zone entourant la tâche cellulaire.
9. Déposer 35 µl de immunoglobulines de mouton anti-lgG de souri conjuguées au FITC dans du bleu Evans (VIR-FITC).
10. Laisser incuber 20 minutes à 37 °C dans une chambre humide.
11. Laver deux fois dans du PBS frais et rincer soigneusement avec de l'eau du robinet (3 fois). Monter dans du milieu de montage et recouvrir d'une lamelle de verre.
12. Effectuer la lecture le plus rapidement possible. Couvrir hermétiquement les lames pour minimiser l'affaiblissement de la coloration.

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Manipulation et stockage

Ils sont livrés dans 0,01 M de phosphate trisodique, 0,15 M NaCl; pH 7,3, 0,2% de bleu Evans, 0,09% de sodiumazide (NaN_3). Stocker les flacons entre 2 et 8 °C. Les fragments de $F(ab)_2$ doivent être protégés d'une exposition prolongée à la lumière. Les réactifs restent stables pour la période indiquée sur l'étiquette du flacon s'ils sont stockés de façon appropriée

Garantie Les produits vendus sont garantis afin d'obéir à la quantité et aux contenus indiqués sur l'étiquette au moment de la livraison au client. Aucune garantie, explicite ou implicite, ne s'étend au-delà de la description de l'étiquette du produit. IQ Products ne peut être tenu responsable des dommages, des blessures ou des pertes économiques provoqués par le produit.

Caractérisation

Afin d'assurer la constante qualité supérieure des réactifs, chaque lot d'anticorps monoclonal est testé afin de vérifier sa conformité aux caractéristiques d'un réactif standard.

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Avertissement

Tous les produits contiennent du sodiumazide. Ce produit chimique est toxique et dangereux. Sa manipulation est réservée au seul personnel formé.

FITC-konjugierte Anti-Maus Immunglobuline vom Schaf

IVD ***In Vitro Diagnostikum***

Dieses Produkt ist nur für die „Nutzung bei In-Vitro-Diagnose" als Teil des CMV Brite™ Turbokit in den Ländern der Europäischen Union registriert. Alle obligatorischen und notwendigen Informationen aus der Richtlinie 98/79/EC für den sicheren und sachgemäßen Umgang mit diesem Produkt befinden sich im Produkthandbuch des CMV Brite™ Turbokit (bitte Anlage beachten).

	Beschreibung
Träger	Schaf
Spezifität	Alle Maus-IgG-Unterklassen
Form	F(ab)-Fragmente reiner FITC-konjugierter Schaf-Immunoglobuline
Anwendungen	Schaf-anti-Maus kann als zweiter Antikörper in Kombination mit dem C10/C11-Cocktail VIR-CMV C10/C11 verwendet werden, um das Untere-Matrix-Protein pp65 (CMV) zu erkennen.
Verwendung	Zur sofortigen Verwendung

Immunfluoreszenzfärbung mit VIR-CMV C10/C11 und VIR-FITC

1. Präparation der Zytocentrifugen-Objektträger (gemäß der Methoden im Labor).
2. Während der Immunfluoreszenzfärbung dürfen die Zellpräparate (Objektträger) nicht austrocknen.
3. Zur Kontrolle der Immunfluoreszenzfärbung können die Kontroll-Objektträger (VIR-CMV CS05), hergestellt durch
   IQ Products, verwendet werden.
4. Kontroll-Objektträger 1-2 Minuten in PBS rehydrieren.
5. Den Objektträger aus der Waschlösung nehmen. Den Bereich um die Zellen vorsichtig trocknen. (Immer nur einen Objektträger entnehmen, um die Zellen vor Austrocknung zu schützen.)
6. 35 µl der C10/C11 monoklonalen Antikörperlösung (VIR-CMV C10/C11) hinzugeben und 20 Minuten bei 37 °C in einer feuchten Kammer inkubieren.
7. Objektträger dreimal für 3 Minuten in Waschlösung (PBS) tauchen.
8. Immer nur jeweils einen Objektträger aus der Waschlösung entnehmen. Den Bereich um die Zellen vorsichtig trocknen.
9. 35 µl der FITC-konjugierten Anti-Maus-Immunglobuline v. Schaf mit Evans Blue (VIR-FITC) hinzugeben.
10. 20 Minuten bei 37 °C in einer feuchten Kammer inkubieren.
11. Objektträger zweimal mit frischem PBS waschen und vorsichtig mit frischem Leitungswasser abspülen (dreimal). Abdecken mit Einschlussmedium und Deckglas.
12. Baldmöglichst auswerten. Objektträger dicht abdecken, um eine Abschwächung der Fluoreszenz zu verhindern

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Handhabung und Lagerung

Sie werden in 0.01 M Natriumphosphat, 0.15 M NaCl; pH 7.3, 0.2% Evans Blue, 0.09% Natriumazid (NaN_3) geliefert. Ampullen bei 2-8 °C lagern. F(ab)-Fragmente sollten vor längerer Lichteinstrahlung geschützt werden. Reagenzien sind bei richtiger Lagerung im auf der Ampulle angezeigten Zeitraum stabil.

Garantie Für die verkauften Produkte gilt eine Garantie nur für die Menge und den Inhalt der auf der Ampulle zum Zeitpunkt der Lieferung an den Kunden angegeben ist. Über die Beschreibung auf der Produktbeschriftung hinaus wird keine ausdrückliche oder implizierte Garantie erteilt. IQ Products ist nicht für Sachschäden, Personenschäden oder wirtschaftliche Verluste verantwortlich, die durch das Produkt entstanden sind.

Beschreibung

Um die gleichbleibenden hohe Qualität der Reagenzien zu bewahren, wird jede Lieferung von monoklonalen Antikörpern auf die Charakteristika eines Standardreagenzes getestet.

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Warnung Alle Produkte enthalten Natriumazid. Dieser chemische Stoff ist giftig und gefährlich. Er sollte ausschließlich von geschulten Personal verwendet werden.

Immunoglobuline anti-mouse sviluppate in pecora marcate con FITC

IVD ***Dispositivo medico-diagnostico in vitro***

Il prodotto è registrato come componente "per esclusivo uso diagnostico in vitro" del kit CMV Brite™ Turbo nei paesi appartenenti alla Comunità Europea. Tutte le informazioni obbligatorie e utili come specificato nella Direttiva 98/78/CE necessarie per l'uso sicuro e adeguato di questo prodotto sono reperibili nel manuale del kit CMV Brite™ Turbo (vedi allegato).

	Descrizione
Ospite	Pecora
Specificità	Tutte le sottoclassi IgG di topo
Forma	Frammenti $F(ab)_2$ di immunoglobuline di pecora purificate coniugate con FITC
Applicazioni	L'antit-mouse sviluppato in pecora può essere utilizzato come secondo anticorpo in combinazione con il mix di C10/C11 VIR-CMV C10/C11 per rilevare la proteina di matrice inferiore pp65 di CMV.
Utilizzo	Soluzione pronta per l'uso

Colorazione immunofluorescente con VIR-CMV C10/C11 e VIR-FITC

1. Preparare i vetrini per la citocentrifuga (come da procedure di laboratorio).
2. Nel corso della colorazione immunofluorescente non lasciar seccare le preparazioni cellulari.
3. Per il controllo della colorazione immunofluorescente è possibile usare i vetrini di controllo (VIR-CMV CS05) prodotti da IQ Products.
4. Reidratare il vetrino di controllo in PBS per 1-2 minuti.
5. Rimuovere il vetrino dalla soluzione di lavaggio. Asciugare con cura l'area che circonda lo spot di cellule. (Rimuovere un vetrino alla volta per evitare che le cellule si essicchino).
6. Applicare 35 µl di soluzione di anticorpi monoclinali (MoAB) C10/C11 (VIR-CMV C10/C11) e incubare a 37 °C per 20 minuti in una camera umida.
7. Immergere i vetrini 3 volte per 3 minuti nella soluzione PBS di lavaggio.
8. Rimuovere un vetrino alla volta dalla soluzione di lavaggio. Asciugare con cura l'area che circonda lo spot di cellule.
9. Applicare 35 µl di immunoglobuline anti-mouse sviluppate in pecora coniugate con FITC con Evans Blue (VIR-FITC).
10. Incubare a 37 °C per 20 minuti in una camera umida.
11. Lavare due volte in PBS fresco e sciacquare abbondantemente con acqua di rubinetto (3 volte). Montare con strumento di supporto e vetro copri oggetto per vetrino da microscopio.
12. Procedere alla lettura appena possibile. Coprire accuratamente i vetrini per ridurre al minimo il calo del colore.

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Manipolazione e conservazione

Sono forniti in 0,01 M fosfato di sodio, 0,15 M NaCl; pH 7.3, 0,2% Evans Blue, 0,09% sodio azide (NaN_3). Conservare le fiale a 2-8 °C. I frammenti $F(ab)_2$ dovranno essere protetti dall'esposizione prolungata alla luce. I reagenti sono stabili per il periodo indicato sull'etichetta della fiala, se conservati correttamente.

Garanzia La sola garanzia offerta è di conformità a quantità e contenuti indicati sull'etichetta al momento della fornitura al cliente. Non vengono concessi altri tipi di garanzia, espressi o impliciti, che esulino dalla descrizione riportata sull'etichetta del prodotto. IQ Products non sarà responsabile di danni alla proprietà, ferite personali o perdite economiche causate dal prodotto.

Caratterizzazione

Per garantire un livello qualitativo costantemente elevato, ciascun lotto di anticorpi monoclonali è testato circa la conformità con le caratteristiche di un reagente standard.

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Attenzione Tutti i prodotti contengono sodio azide, sostanza chimica velenosa e pericolosa. L'uso sarà consentito esclusivamente a operatori specializzati.

Imunoglobulinas de ovelha anti-rato conjugadas com FITC

IVD ***Dispositivos médicos de diagnóstico in Vitro***

Este produto está registado "apenas para fins de diagnóstico *in vitro*" como componente do CMV Brite™ Turbo kit em países que pertencem à Comunidade Europeia. Todas as informações relevantes e obrigatórias, tal como especificadas na Directiva 98/79/EC, necessárias para a utilização segura e correcta deste produto, podem ser encontradas no manual do produto CMV Brite™ Turbo kit (consulte anexo).

	Descrição
Anfitrião	Ovelha
Especificidade	Todas as subclasses IgG de rato
Forma	Fragmentos $F(ab)_2$ de imunoglobulinas de ovelha purificadas conjugadas com FITC
Aplicações	O anticorpo de ovelha anti-rato pode ser utilizado como um segundo anticorpo combinado com o *cocktail* de C10/C11 (VIR-CMV C10/C11) para detectar a pp65 (proteína da matriz inferior do CMV)
Utilização	Solução pronta a utilizar

Coloração de imunofluorescência com VIR-CMV C10/C11 e VIR-FITC

1. Preparar lâminas de citocentrifugação (de acordo com os procedimentos laboratoriais).
2. Durante a coloração de imunofluorescência não permitir a secagem das preparações de células.
3. Para controlar a coloração de imunofluorescência, é possível utilizar lâminas de controlo (VIR-CMV CS05) produzidas pela IQ Products.
4. Hidratar novamente a lâmina de controlo em PBS durante 1 - 2 minutos.
5. Remover a lâmina da solução de lavagem. Secar cuidadosamente a área em torno da célula. (Remover uma lâmina de cada vez para evitar a secagem das células.)
6. Aplicar 35 µl de solução MoAb C10/C11 (VIR-CMV C10/C11) e incubar durante 20 minutos a 37 °C numa câmara húmida.
7. Mergulhar as lâminas 3 vezes numa solução de lavagem PBS durante 3 minutos.
8. Remover uma lâmina de cada vez da solução de lavagem. Secar cuidadosamente a área em torno da célula.
9. Aplicar 35 µl de imunoglobulinas de ovelha anti-rato conjugadas com FITC e Azul de Evans (VIR-FITC).
10. Incubar durante 20 minutos a 37 °C numa câmara húmida.
11. Lavar duas vezes em PBS novo e lavar cuidadosamente com água da torneira (3 vezes). Colocar num suporte para lâminas e colocar uma lamela.
12. Efectuar a leitura assim que possível. Cobrir bem as lâminas para minimizar a descoloração.

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Instruções e Armazenamento

São fornecidos em 0.01 M de fosfato de sódio, 0.15 M NaCl; pH 7.3, 0.2% de Azul de Evans, 0.09% de azida de sódio (NaN_3). Os frascos devem ser armazenados entre 2-8 °C. Os fragmentos $F(ab)_2$ devem ser protegidos de exposição prolongada à luz. Os reagentes permanecem estáveis durante o período indicado no rótulo do frasco, desde que sejam correctamente armazenados.

Garantia A garantia dos produtos vendidos ao abrigo da mesma abrange apenas a quantidade e os conteúdos mencionados no rótulo aquando da entrega ao cliente. Não existem quaisquer garantias, expressas ou implícitas, que abranjam mais do que o descrito no rótulo do produto. A IQ Products não é responsável por quaisquer danos de propriedade, danos pessoais ou perdas económicas causadas pelo produto.

Caracterização

Para garantir a consistência de reagentes de alta qualidade, cada lote de anticorpos monoclonais é testado em conformidade com as características de um reagente padrão.

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Aviso Todos os produtos contêm azida de sódio. Este químico é venenoso e perigoso. O manuseamento deve ser efectuado apenas por pessoal técnico qualificado.

Anticuerpo de oveja inmunoglubinas de ratón conjugadas en FITC

IVD ***Producto sanitario para diagnóstico in vitro***

Este producto está registrado para "uso exclusivo en diagnóstico in-vitro" como un componente del kit CMV Brite™ Turbo en los países miembros de la Unión Europea. Toda la información obligatoria y necesaria según las especificaciones de la Directiva 98/79/EC para el uso seguro y apropiado de este producto se puede encontrar en el manual del producto del Kit CMV Brite™ Turbo (por favor, consulte el documento adjunto).

	Descripción
Portador	Oveja
Especialidad	Todos los subconjuntos de IgG ratón
Forma	fragmentos $F(ab)_2$ de inmunoglobulinas de oveja purificadas y conjugadas en FITC
Aplicaciones	Anticuerpo de oveja antiglobulina de ratón se puede utilizar como un segundo anticuerpo en combinación con la mezcla C10/C11 de VIR-CMV C10/C11 para detectar proteína pp65 de matriz baja CMV
Uso	Solución lista para su uso

Tintado inmunofluorescente con VIR-CMV C10/C11 y VIR-FITC

1. Prepare las láminas cito centrífugas (de acuerdo con los procedimientos de laboratorio).
2. No deje que las preparaciones de las células se sequen durante el tintado inmunofluorescente.
3. Para controlar el tintado inmunofluorescente puede utilizar láminas de control (VIR-CMV CS05) producidas por IQ Products.
4. Vuelva a hidratar la lámina de control en PBS durante 1-2 minutos.
5. Retire la lámina de la solución de limpieza. Seque con cuidado la zona alrededor del lugar de la célula. (Retire cada lámina una a una para evitar que se sequen las células.)
6. Aplique 35 µL de solución Mab (anticuerpo monoclonal) (VIR-CMV C10/C11) e incúbela durante 20 minutos en una cámara húmeda a 37 °C.
7. Sumerja las láminas 3 veces en una solución de lavado PBS durante tres minutos.
8. Retire cada lámina una a una de la solución de limpieza. Seque con cuidado la zona alrededor del lugar de la célula.
9. Aplique 35 µL de anticuerpo de oveja inmunoglobulinas de ratón conjugadas FITC con Evans Blue (VIR-FITC).
10. Incube durante 20 minutos a 37 °C en una cámara húmeda.
11. Lávelo dos veces en una solución PBS recién preparada y enjuáguelo con cuidado con agua del grifo (3 veces). Móntelo con dispositivo de montaje y cristal de microscopio.
12. Efectúe la lectura tan pronto como sea posible. Ajuste bien la cubierta de las láminas para que no se disuelva.

Tratamiento y almacenamiento

Se presentan con un fosfato de sodio de 0.01 M, 0.15 M NaCl; pH 7.3, 0.2% Evans Blue, 0.09%, azida de sodio (NaN3). Guarde los frascos a una temperatura de entre 2-8 °C. Se deberían proteger los fragmentos $F(ab)_2$ de la exposición prolongada a la luz. Los reactivos son estables durante el periodo mostrado en la etiqueta del frasco si se almacenan como es debido.

Garantía Los productos que se venden a continuación tienen una garantía conforme a la cantidad y los contenidos que se establecen en la etiqueta en el momento de la entrega al cliente. No existen garantías, explícitas o implícitas que se extiendan más allá de la descripción de la etiqueta del producto. IQ Products no se responsabiliza de cualquier daño personal o material o pérdida económica causada por el uso del producto.

Caracterización

Para asegurar unos reactivos consistentes y de alta calidad, cada serie de anticuerpos monoclonales se pone a prueba para conformarse a las características de un reactivo estándar.

Aviso Todos los productos contienen azida de sodio. Este compuesto químico es venenoso y peligroso. Solamente un personal entrenado debe manejarlo.

IQ Products BV
Rozenburglaan 13a, 9727 DL Groningen, The Netherlands
☏ +31 (0)50 57 57 000
🖷 +31 (0)50 57 57 002
Information marketing@iqproducts.nl
Orders orders@iqproducts.nl
Tech support techsupport@iqproducts.nl
www.iqproducts.nl

Attachment : Package Insert - CMV Brite™ Turbo Kit

Rapid CMV pp65 Antigenemia for the detection of active CMV infection

REF[9] VIR-CMV 110 ∇ 110 Tests IVD CE0344

Intended use

The CMV Brite™ Turbo Kit is intended for the rapid qualitative detection of Cytomegalovirus (CMV) lower matrix protein pp65 by indirect immunofluorescence using microscopy in isolated peripheral blood leukocytes obtained from ethylenediaminetetraacetic acid (EDTA) or heparin anti-coagulated human peripheral blood. The detection of CMV pp65 in human peripheral blood cells aids in the diagnosis of acute or reactivated CMV infection. This product is not FDA cleared (approved) for use in testing (i.e. screening) of blood or plasma donors.

Summary and Explanation

Infections with human cytomegalovirus (CMV), a β-herpes virus, are widespread throughout the world (rate of prevalence 40-100%). While an infection with CMV proceeds asymptomatically in the majority of immuno-competent persons, it can lead to serious complications in persons whose immune system has been weakened or is not yet fully developed (hepatitis, retinitis, pneumonie etc.). CMV is the most frequent pathogen of congenital infections. Approximately 10% of children congenitally infected with CMV show symptoms at birth (icterus, hepatosplenomegaly, petechial bleeding and chorioretinitis). Further groups of patients for whom an acute CMV infection represents a serious threat are recipients of organ and bone-marrow transplants and AIDS patients. The CMV antigenemia assay has been developed using a cocktail of two monoclonal antibodies (C10/C11) directed against pp65 [2]. The CMV antigenemia assay is valuable in the diagnosis of active CMV infection. Shell vial cultures provide a result within 1 to 2 days, but are not sensitive for detection of CMV in blood specimens. In contrast, detection of CMV antigen in peripheral blood polymorphonuclear (PMN) cells (CMV antigenemia) is both sensitive and rapid [1, 2]. This technique uses monoclonal antibodies to detect the CMV lower matrix phosphoprotein (pp65), an early antigen in virus replication, that is abundantly present in antigen-positive PMNs [2-5]. The CMV Brite Turbo assay can be completed within 2 hours of sample collection.

Principle of the test - immunofluorescence test -

The CMV Brite™ Turbo method consists of:

a. direct lysis of peripheral blood erythrocytes
b. preparation of cytospin slides
c. fixation and permeabilization
d. indirect immunofluorescence staining using monoclonal antibodies directed against CMV pp65 protein.
e. reading and evaluation of results

The first step in the CMV Brite™ Turbo method involves direct lysis of the peripheral blood erythrocytes [6]. Following lysis the leukocytes are cytocentrifuged onto a slide, fixed and permeabilized to allow subsequent detection of pp65 antigen. The presence of pp65 is detected by the C10/C11 antibody cocktail and visualized by means of a specific secondary FITC-labeled antibody. CMV antigen-positive leukocytes exhibit homogeneous yellow-green polylobate nuclear staining when observed using a fluorescence microscope. The number of CMV antigen-positive cells is counted per duplicate stain.

Kit contains

LYS	200 ml	**Reagent A (A)**, Erythrocyte lysing solution (Ammonium chloride solution, sodium azide < 0,1%) Concentrate: dilute 1:10 with demineralized water  WARNING
FIX	290 ml	**Reagent B (B)**, Fixative solution (formaldehyde in PBS, sodium azide < 0,1%) Concentrate: dilute 1:5 with PBS  DANGER
PER	290 ml	**Reagent C (C)**, Permeabilization solution (Igepal Ca-630, newborn calf serum in PBS, sodium azide < 0,1%), Concentrate: dilute 1: 5 with PBS
MAB	4 ml	**Reagent D (D)**, Monoclonal antibody (mouse), Mix of C10/C11 (IgG_1/IgG_1) against lower matrix protein pp65. sodium azide < 0,1% (Ready to use)
CONJ	4 ml	**Reagent E (E)**, FITC-conjugated sheep anti-mouse-immunoglobulins with Evans Blue, sodium azide < 0,1%. (Ready to use)
CONTR	5 x 1	**Control Slide**, CMV antigenemia control microscope slides. Control slide in a sealed pouch with desiccant. (Ready to use)

Laboratory material required and not included in the Kit

Laboratory centrifuge; 50 ml conical bottomed centrifuge tubes; Sterile pipettes, micropipette and tips; phosphate buffered saline (PBS), pH 7.4, Ca, Mg free; Hemocytometer or automated cell counter; Cytocentrifuge slides; Cytocentrifuge (such as Shandon Southern Products, Ltd., model Cytospin 3); Coplin jars or histology staining jars; Humid chamber; 37 °C incubator; Fluorescence microscope capable of 250x to 1000x magnification; Mounting medium (non-fluorescent, such as Citifluor, Glycerol PBS solution, UKC Chem Lab; or Immunoconcept Mounting Medium, Catalogue # 111); Fume Hood; Micro cover glass; Stop watch/Timer.

Warning and precautions

Do not incorporate reagents. Avoid contact with eyes and skin. All samples and materials used for the test must be treated as being potentially infectious and appropriate safety precautions taken. In the preparation of the CMV Brite™ Turbo Control Slides, leukocytes have been used obtained from a healthy human blood donor. Each donor sample has been tested and found non-reactive for the presence of antibodies to HIV-1 , HIV-2, HCV and CMV as well as for HBsAg. Do not pipette with mouth. According to good laboratory practice wear gloves, laboratory coat and safety glasses. Liquids and non-combustible materials should be decontaminated with sodium hypochlorite (final concentration: 3%, activity time at least 30 minutes). Liquid waste which contains acids must be neutralized before disposal. All materials that are to be reused must be autoclaved for 1 hour at 121 °C. LYS contains Ammonium chloride. H302 Harmful if swallowed; H319 Causes serious eye irritation. P305 + P351 + P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. FIX contains less than 9.3% formaldehyde. Formaldehyde is a highly toxic allergenic and potentially carcinogenic reagent, which should be handled in accordance with good laboratory practices using appropriate precautions. Avoid skin or eye contact. H301 + H311 + H331 Toxic if swallowed, in contact with skin or if inhaled; H314 Causes severe skin burns and eye damage; H317 May cause an allergic skin reaction; H335 May cause respiratory irritation; H351 Suspected of causing cancer; H370 Causes damage to organs. P260 Do not breathe dust/ fume/ gas/ mist/ vapours/ spray;P280 Wear protective gloves/ protective clothing/ eye protection/ Face protection; P301 + P310 IF SWALLOWED: Immediately call a POISON CENTER or doctor/physician; P305 + P351 + P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing; P310 Immediately call a POISON CENTER or doctor/ physician. CONJ contains Evans Blue. Avoid contact with eyes, skin, clothing. Keep container tightly closed. Wash thoroughly after contact with FIX and CONJ and consult a doctor. The test must be performed by well-trained and authorized laboratory technicians. Testing is performed under aseptic and microbiologically controlled conditions. Please contact the manufacturer if the original test kit is damaged.

Storage

Upon receipt, store reagents at 2-8 °C. Avoid direct sunlight. Reagents stored according to stated storage instructions are stable until the expiration date indicated on the label. For repeatedly testing store the reagents immediately after usage at 2-8 °C.

Processing of the blood sample

Collect between 3 to 5 ml venous blood into an EDTA-treated tube, using aseptic venipuncture. Send the sample to the laboratory without delay. The blood sample should be kept at room temperature (20-25 °C) until processing. Processing should be performed within 6 to 8 hours of sample collection since it has been shown that a decrease of antigen-positive cells (collected in heparin) can be found after storage [7-8]. Therefore, immediate preparation of the slides should always be attempted. In patients with severe neutropenia (absolute neutrophil count less than 200/mm^3) at least 10 ml of blood may be required. If samples are to be transported, they must be packed in accordance with legal requirements for the transportation of infectious materials.

Test Procedure

The protocol has to be followed strictly. Unless stated otherwise, reagents (including PBS) should be at room temperature when used in the test procedure. Room temperature is defined as 20-25 °C.

I. Preparation of leukocyte suspension
- Dilute Reagent A 1:10 in demineralized (distilled) water and allow to cool to 2-8 °C.
- Mix 2 ml blood with 30 ml of cold (+4 °C) diluted erythrocyte lysing solution in a 50 ml conical bottomed tube and incubate for 5 minutes at 2-8 °C.
- Centrifuge for 2 minutes at 1000xg (2500 rpm). Discard the supernatant.
- Repeat the cold lysing step if lysing of erythrocytes is not sufficient after the first time.
- Resuspend the cell pellet in 30 ml PBS.
- Centrifuge for 2 minutes at 1000xg (2500 rpm). Discard the supernatant
- Resuspend the pellet in 1 ml PBS. (In patients with severe neutropenia re-suspension in as low as 0.2 ml may be required).

II. Cell counting
- Count cells using a Hemocytometer or automated cell counter.
- Adjust concentration to 2.0 x 10^6 cells/ml by diluting in PBS.

III. Preparation of cytocentrifuge slides
- Centrifuge 100 µl of the 2.0 x 10^6 cells/ml suspension at approx. 600 rpm (54x g) for 4 minutes onto glass slides by cytocentrifuge.
- Prepare at least 3 slides per patient specimen (2 for testing, plus an additional slide for backup).
- Let slides dry for approximately 5 minutes.
- Circle cell area on the slide using a laboratory marker pen.
- Slides can be kept at room temperature overnight before fixation.

IV. Fixation and permeabilization
- Dilute fixative (reagent B) 1: 5 in PBS in a fume hood prior to use.
- Dilute permeabilization solution (reagent C) 1: 5 in PBS in a fume hood prior to use. (Do not reuse.)
- Immerse 2 slides in diluted reagent B for 5 minutes at room temperature in the fume hood.
- Dip slides 3 times in PBS (washing solution) and leave in the washing solution for 3 minutes.
- Immerse slides in diluted reagent C for 1 minute at room temperature.
- Dip slides 3 times in washing solution and place in fresh washing solution for 5 minutes (or any time up to 60 minutes). If staining with monoclonal antibody is to follow directly then proceed to step V.
  To store slides:
  If slides are to be stored then rinse in demineralized (distilled) water for 15 seconds. Let slides dry under the ventilator for approximately 20 minutes. Once dry, slides should be packed in aluminum foil and stored at 2-8 °C for 24 hours, or frozen at -80 °C.

V. Immunofluorescence staining
- From this point on, do not allow the cell preparations to dry out during the remainder of the staining procedure.
- CMV Brite™ Turbo Control slide. Rehydrate control slide in PBS for 1 or 2 minutes.
- Remove one slide at a time from the washing solution, carefully dry the area surrounding the cell spot.
- Apply 35 µl of C10/C11 MoAb solution (reagent D) incubate for 20 minutes at 37 °C in a humid chamber.
- Dip slides 3 times in washing solution (PBS) and put in fresh washing solution for 3 minutes.
- Remove one slide at a time from the washing solution, carefully dry the area surrounding the cell spot.
- Apply 35 µl conjugate (reagent E), incubate for 20 minutes at 37 °C in a humid chamber.
- Wash twice in fresh PBS and carefully rinse with tap water (3 times) and mount with mounting medium and a micro cover glass.

VI. Reading
Perform reading as soon as possible. Slides may be stored for up to 8 hours at 2-8 °C covered tightly in order to minimize fading. Perform microscopic evaluation using an immunofluorescence microscope at 400x magnification. A higher magnification of 1000x may be used to increase resolution. (See note on recommendations for microscope and fluorescence cube under "Laboratory material and equipment".) Scan the whole surface of the spot. Two spots should be scanned per patient. Positive cells show homogenous yellow-green polylobate nuclear staining. Negative cells show no yellow-green nuclear staining. Equivocal readings due to artifacts may occur rarely (less than 5%).
The most common artifact is due to eosinophils. They are recognized by a nonspecific cytoplasmatic staining, with the nucleus appearing as black holes, often spectacle-shaped. The cytoplasm may appear dull and more yellow. If all cells appear greenish, this represents an artifact, which may be associated with some types of patients and is very rare.
A greenish staining of cells at the periphery of the spot only, is not considered positive. This may occur if the spot has started to dry out during incubation with either monoclonal antibody solution or conjugate solution.
Be sure to incubate in a humid chamber and check that the whole cell spot is covered with the reagent.
If slides are not interpretable due to equivocal readings of artifacts, stain backup slide(s) or if unresolved obtain and test an additional specimen.

Quality Control
The positive and negative control slides are provided with the kit. The slides are only used to check the staining procedure and do not influence the diagnostic value of the kit. The positive control must demonstrate appropriate staining before patient specimens can be evaluated. The positive control should exhibit homogeneous yellow-green staining of positive cells with round morphology (nucleus is not visible). The negative control should show no yellow-green staining. Despite the fact that the quality procedures are followed strictly, single positive cells could be observed in the negative control. In such cases the staining run should **not** be considered invalid. The single positive cells on the negative control do not mean that the slide with patient material cannot be interpreted.

Choice of microscope
Selecting the correct filter cube configuration, which matches the fluorochrome in use, is important. This will vary for each microscope manufacturer. Select the correct combination for FITC use, such as the Olympus BX-40 or BX-60 microscope with: Fluorescence cube (U-MNB), Excitation wavelength (470-490 nm), Emission wavelength (> 515 nm).

Reading and interpretation of result
Results of evaluation of patient specimen slides are qualitative.
Positive result: one or more CMV antigen-positive cells present per duplicate stain. Negative result: no CMV antigen-positive cells present per duplicate stain. A minimum of approximately 50,000 specimen cells should be present in order to determine that a result is negative. If equivocal readings due to artifacts occur on both duplicate stains, the results should not be interpreted. Stain back-up slides or obtain and test an additional patient specimen.

Limitations of the procedure
Detection of CMV pp65 lower matrix, early structural protein should be performed by laboratories experienced in immunocytochemical techniques.
Leukocyte preparation should be performed by personal experienced in aseptic techniques. The efficacy of the CMV Brite™ Turbo Kit with samples other than human blood leukocytes has not been established. Test performance characteristics have been established using the CMV Brite™ Kit (EDTA and heparin specimens) and validated in internal studies using the CMV Brite™ Turbo Kit (EDTA specimens only). The CMV Brite™ Turbo Kit is intended for use only with immunofluorescent microscopy and not for use with flow cytometry. The type and condition of the instrumentation used will influence the visual appearance of the image obtained. The reaction may vary due to the type of microscope employed, the light source, age of the bulb, filter assembly and filter thickness, differences in sensitivity of the antigen substrate, or the assay procedure used. Each laboratory should establish its own criteria for the reading of patient specimens using appropriate controls. The detection and confirmation of CMV pp65 lower matrix, early structural protein in peripheral blood leukocytes is not diagnostic of symptomatic illness, since CMV pp65 may be present for a significant period following acute infection and patients with CMV antigenemia (especially low levels) may have asymptotic illness. The are many published reports of patients who are antigenemia-negative and viremia-positive and some of these patients may have CMV disease. Thus, a negative antigenemia test does not absolutely exclude CMV infection or disease. The CMV Brite™ Turbo Kit is not intended for antiviral drug monitoring.

Test performance characteristics have not been established using neonatal specimens. A decrease can be noted of antigen-positive cells (collected in heparin) per slide after storage [7-8]. Therefore, immediate preparation of the slides should always be attempted. The maximal specimen storage time at room temperature has not been determined. In patients with severe neutropenia (absolute neutrophil count less than 200/mm^3) at least 10 ml of blood may be required. Since the monoclonal antibodies have been prepared using a prototype strain, they may not detect all antigenemic or new strains of CMV. For example, monoclonal antibodies may fail to detect strains of CMV which have undergone minor amino acid changes in the target epitope region. Test performance characteristics have not been established using specimens collected in heparin.

Performance characteristics
The CMV Brite™ Kit was compared to CMV virus detection in conventional culture (CC) and shell vial culture (SV) using human peripheral blood leukocytes. 300 clinical samples were evaluated in comparison to conventional culture (CC) and shell vial culture (SV). A total of 300 venous blood samples were collected from transplant recipients (159 allogeneic bone marrow and 47 solid organ), 85 HIV positive/AIDS patients, and 9 immunocompetent patients. 92 samples (30.7%) were determined to be CMV positive by the CMV Brite™ Kit. In random blood donor populations, the number of specimens found repeatedly reactive for CMV antigenemia by the CMV Brite™ Kit has typically been 0%. Note: The performance characteristics have been established using the CMV Brite™ Kit and validated in internal studies using the CMV Brite™ Kit Turbo Kit.

Comparison of CMV Brite™ Kit with CC and SV

	CMV Brite Kit		
CC/SV	+	-	total
+	31	3	34
-	61	205	266
total	92	208	300

Sensitivity: 31/34 = 91.2% (95% CI = 76.3 to 98.1%)
Specificity: 205/266 = 77.1% (95% CI = 70.2 to 81.1%)

The performance of the CMV Brite™ Turbo Kit was validated in a study of 183 patient samples. The patient samples included 173 organ transplant patients, 9 immunocompetent patients and 1 HIV positive patient sample. Each patient was tested in parallel with the CMV Brite™ and the CMV Brite™ Turbo Kits. The results of this validation study are provided in the table below.

	CMV Brite™ Kit		
CMV Brite™ Turbo Kit	+	-	Total numbers
+	43	7	50
-	6	127	133
Total numbers	49	134	183

Cross reactivity
For the analysis of cross reactivity clinical isolates of the following viruses were tested: Herpes virus type 1 and type 2, Varicella-zoster virus, Adenovirus 2, 4 and 5, Parainfluenza virus 1, 2 and 3, Respiratory syncytial virus, Poliovirus 3 (wild type 3), Echovirus 11, Echovirus 30, Epstein-Barr virus (laboratory strain P3HR1) , Human herpes virus type 6 (laboratory strain GS) , Human immunodeficiency virus (type 1 commercially available IF slides) . No cross reactivity was observed except for a weak positive reaction with six of the seven HSV-1 isolates in the shell vial assay. The weak staining observed with the six HSV-1 isolates was cytoplasmic (i.e., seen only outside the nucleus, in a limited number of small foci), similar to the IF pattern usually found with HSV-1 monoclonal antibodies. The weak staining observed may have been due to Fc receptors being expressed by the HSV-1 infected cells in the shell vial assay. Subsequent analysis concluded that there is no evidence of cross reactivity between HSV and the CMV antigenemia assay using the C10/C11 monoclonal antibody cocktail.

Literature

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9. NEN EN ISO 15223-1 Medical devices - Symbols to be used with medical device labels, labeling and information to be supplied – Part 1: General requirements.

Explanation of used symbols

	Consult instructions for use
REF	Catalogue number
	Sufficient for
IVD	In Vitro Diagnostic medical device
	Caution, consult accompanying document
	Keep away from (sun)light
	Biological risks
	Temperature limitation (°C)
RUO	For Research Use Only
LOT	Batch code
	Use by yyyy-mm-dd
	Manufacturer
EC REP	Authorized Representative in the European Community
CE	Conformité Européenne (European Conformity)