

Microscopy Tools III: ONCYTE® MultiWells and Slides

Table 1. ONCYTE® nitrocellulose coated slides.

Product	Dimensions*	Wells	Quantity per Package
ONCYTE® MultiWells**			
O24750	5 mm diameter	12	20
O24751	13 mm diameter	1	20
ONCYTE® NOVA nitrocellulose slides			
O24752	51 mm × 20 mm	1 pad	20
O24753	6 mm × 6 mm	16 pads	20
ONCYTE® AVID nitrocellulose slides			
O24754	51 mm × 20 mm	1 pad	20
O24755	6 mm × 6 mm	16 pads	20
*Dimensions are listed for the nitrocellulose pad. **Includes matching gaskets.			

Introduction

Invitrogen, in association with Grace Bio-Labs, provides exciting new tools for microscopy and imaging applications. The ONCYTE® nitrocellulose product is a three-dimensional microarray substrate bound to a standard 25 mm × 75 mm glass slide. A combination of nitrocellulose polymer and proprietary chemistry maximizes protein binding and stability. The ONCYTE® nitrocellulose products are available in three formats for your convenience; ONCYTE® MultiWells, ONCYTE® NOVA nitrocellulose coated slides, and ONCYTE® AVID nitrocellulose coated slides.

ONCYTE® MultiWells

The versatile ONCYTE® MultiWells consist of a two-piece set that includes a slide printed with nitrocellulose circles and a matching removable gasket to enclose and isolate each sample (Figure 1, Table 1). The nitrocellulose coating on the slide is specially formulated for fluorescent imaging. This ultra-thin microporous coating ensures uniform binding of tissue prints, cells or macromolecules, and becomes transparent in a variety of mounting media. The matching press-to-seal silicone gaskets adhere easily to the surface of the slide to isolate specimens and reagents and prevent cross contamination. A coverslip can be added to create enclosed chambers for long incubations. Gaskets can be removed cleanly and easily, simply by peeling them off. The ONCYTE® MultiWells with 12 wells are ideal for imaging of multiple samples in a high-throughput format.

ONCYTE® NOVA Nitrocellulose Slides

ONCYTE® NOVA nitrocellulose slides (Figure 2, Table 1) enable the greatest fluorescence sensitivity. The three-dimensional microarray substrate is bound to a standard 25 mm × 75 mm glass slide. A combination of nitrocellulose polymer and proprietary chemistry maximizes protein binding and stability. The film is compatible with virtually all detection systems, including fluorescent, chemiluminescent, radiographic, and colorimetric as well as reagents used in most cell analysis assays including 50% formamide. Among other applications, it is used for protein microarrays, immunoarrays, antibody arrays, analyte microarrays, cell lysate arrays, and tissue printing. The ONCYTE® NOVA nitrocellulose slide is recommended for applications that require the greatest fluorescence sensitivity.

ONCYTE® AVID Nitrocellulose Slides

ONCYTE® AVID nitrocellulose slides (Figure 2, Table 1) enable the highest possible binding capacity and affinity. The three-dimensional microarray substrate is bound to a standard 25 mm × 75 mm glass slide. A combination of nitrocellulose polymer and proprietary chemistry maximizes protein binding and stability. The film is compatible with virtually all detection systems, including fluorescent, chemiluminescent, radiographic, and colorimetric as well as reagents used in most cell analysis assays including 50% formamide. Among other applications, it is used for protein microarrays, immunoarrays, antibody arrays, analyte microarrays, cell lysate arrays, and tissue printing. The ONCYTE® AVID nitrocellulose slide is recommended for applications that require the highest possible binding capacity and affinity.

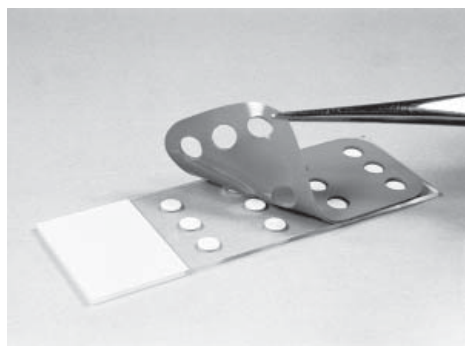


Figure 1. ONCYTE® MultiWells with the 12 nitrocellulose circles and matching gasket to form 12 sample wells.



Figure 2. ONCYTE® nitrocellulose coated slides with 1 pad or 16 pads.

How to Use

ONCYTE® MultiWells

- 1.1** Using a forceps, position the gasket so that the openings are in register with the printed nitrocellulose circles on the slide. Lay the gasket on the slide and press to seal.
- 1.2** Pipet the sample into the wells formed by the gaskets. Due to the hydrophobic nature of the silicone, the volume added can exceed the well volume. For volumes less than the well volume, a glass coverslip can be added to the top to create an enclosed chamber.
- 1.3** To remove the silicone isolator, simply peel it off.

ONCYTE® Slides

The ONCYTE® nitrocellulose slides are used for protein microarrays, immunoarrays, antibody arrays, analyte microarrays, cell lysate arrays, and tissue printing. An immunohistochemical staining procedure is described below. For detailed protocols on other applications, refer to published references (Cell Vision 2, 499, (1995); BioTechniques 20, 641 (1991); Cancer Res 67, 6247 (2007); Blood 108, 1668 (2006)).

Immunohistochemical Staining Protocol

- 2.1 Apply specimen to the nitrocellulose area by any conventional method including centrifugation, cryostat mounting, tissue printing, and array depositors. Soluble analytes maybe bound to the slide by air drying, UV crosslinking, baking, or microwave. Air dry the samples.
- 2.2 Fix specimen at 4°C using appropriate fixative (such as 4% paraformaldehyde in phosphate buffered saline or compatible alcohols) for the applied antigen. Fixation time may vary according to the specimen thickness and specific antigen.

Note: The slides are not compatible with acetone, ethanol, methanol but are compatible with isopropanol and butanol.

- 2.3 Wash the specimen 3 times with 1X PBS (phosphate buffered saline) containing 0.1% Tween 20 (TPBS).
- 2.4 Block non-specific primary antibody binding using appropriate blocking reagent as follows:
 - Peroxidase based detection: Incubate specimens for 15 minutes at room temperature with 0.3% hydrogen peroxide in deionized water.
 - Alkaline phosphatase detection: Incubate specimens for 15 minutes at room temperature in TPBS with 1% normal serum from the species in which the secondary antibody is prepared.
- 2.5 Blot excess serum from specimens.
- 2.6 Incubate specimens for 30 minutes with primary antibody diluted in TPBS containing 1% normal serum.
- 2.7 Wash specimens 3 times with TPBS.
- 2.8 Incubate specimens for 30 minutes with secondary antibody diluted in TPBS containing 1% normal serum.
- 2.9 Wash specimens 3 times with TPBS.
- 2.10 Proceed to the appropriate detection step (alkaline phosphatase detection steps are described below):
 - a) Incubate specimens in alkaline phosphatase labeled reagent.
 - b) Wash specimens 3 times with TPBS.
 - c) Equilibrate specimens for 2 minutes in 0.1 M Tris, pH 9.5.
 - d) Incubate in substrate solution for 20–30 minutes.
 - e) Rinse with water.
- 2.11 Counterstain, if desired. (ONCYTE® slides are compatible with a variety of counterstains including Mayer's, hematoxylin, nuclear fast red and light green SF.)
- 2.12 Air dry or dehydrate through isopropanol series.
- 2.13 Clear with 3 changes of xylenes. Mount using non-aqueous medium.

Product List Current prices may be obtained from our website or from our Customer Service Department.

Cat. no.	Product Name	Unit Size
O24750	ONCYTE® MultiWells, 12 wells, 5 mm diameter, with slide and matching gasket *set of 20*	1 set
O24751	ONCYTE® MultiWells, one well, 13 mm diameter, with slide and matching gasket *set of 20*	1 set
O24752	ONCYTE® NOVA Nitrocellulose coated slides, 1 pad, 51 mm × 20 mm, frosted end slide *set of 20*	1 set
O24753	ONCYTE® NOVA Nitrocellulose coated slides, 16 pad, 6 mm × 6 mm *set of 20*	1 set
O24754	ONCYTE® AVID Nitrocellulose coated slides, 1 pad, 51 mm × 20 mm, frosted end slide *set of 20*	1 set
O24755	ONCYTE® AVID nitrocellulose coated slides, 16 pad, 6 mm × 6 mm, frosted end slide *set of 20*	1 set

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