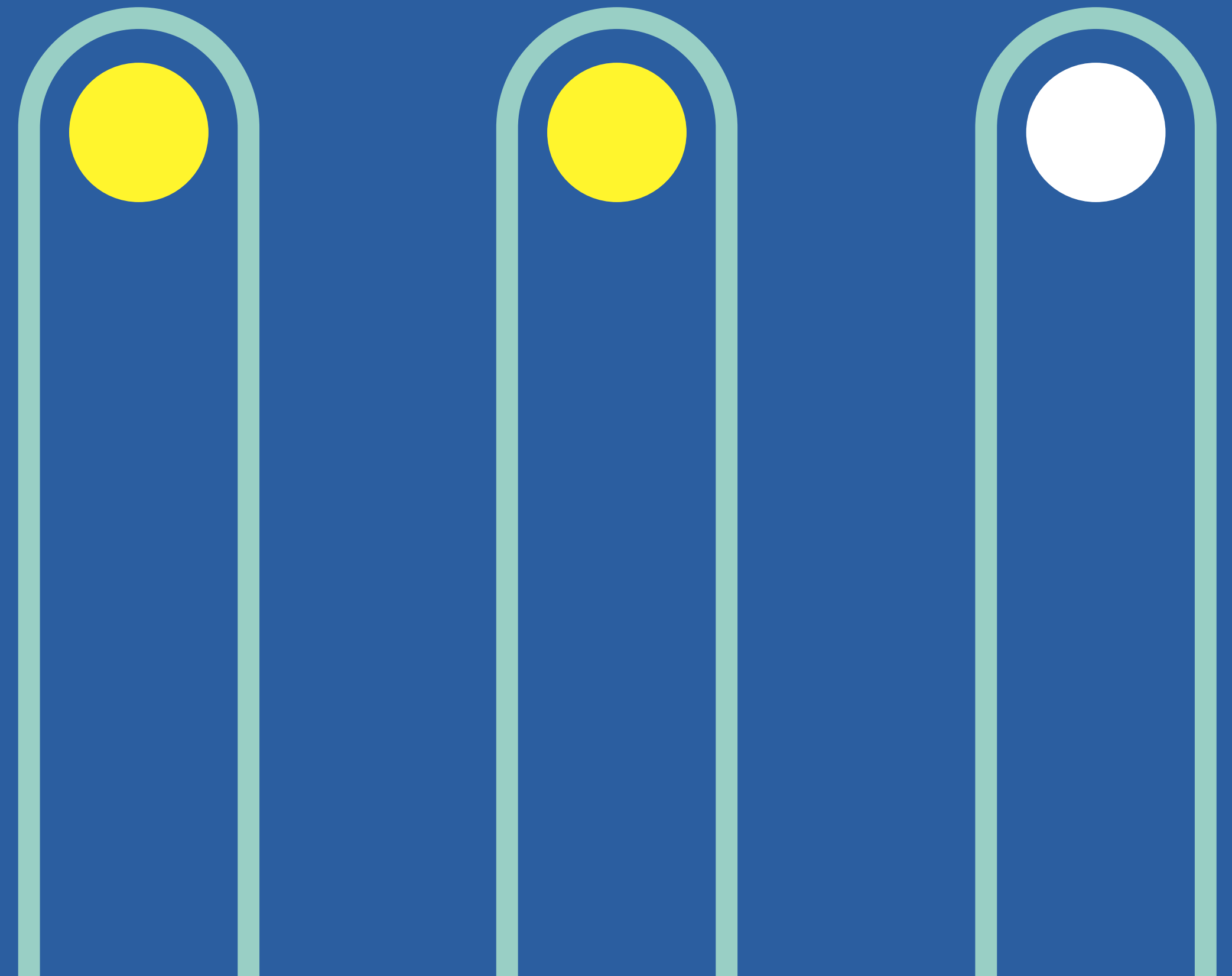


# Whatman point-of-care testing

Components for diagnostic assay design: blood



# The sensitivity and specificity of your assay are only as good as its components

Your ability to quickly and reliably deliver product to market is only as strong as the partners in your supply chain. Whether we are supporting you in development of point-of-care tests or lab-based assays, Cytiva focuses on producing reliable components with highly reproducible performance that are delivered on time, every time. For assays requiring the highest level of quality assurance, we can provide diagnostics products manufactured to ISO 13485:2016 upon request.

To further help with your design, this guide provides general advice on component selection. If you'd like a bit more help please contact us for a design consultation at [Diagnostics.DesignServices@cytiva.com](mailto:Diagnostics.DesignServices@cytiva.com)

# Contents

**01**    **Point-of-care  
immunoassays**            **pg 4**

**02**    **Antibody purification**            **pg 21**

**03**    **Design services**            **pg 24**

# 01

## Point-of-care immunoassays

Rapid point-of-care tests are among the most widely used analytical technologies in diagnostics. Due to their high performance, ease of use and cost effectiveness, diagnostic rapid tests can quickly deliver reliable results in challenging settings.

Cytiva is an established technology component provider for point-of-care immunodiagnostic and enzymatic assays, specifically:

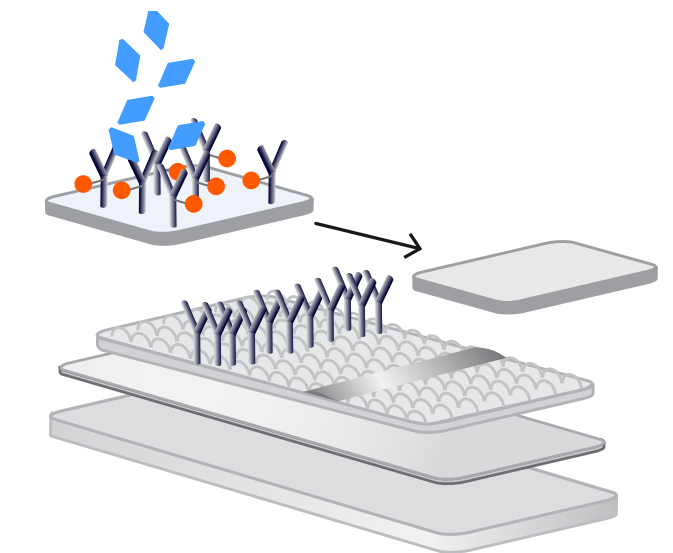
- Lateral flow immunoassays
- Flow-through immunoassays
- Dipstick colorimetric assays

We produce a comprehensive range of cellulose and glass fiber substrates and nitrocellulose membranes to an assured quality, ensuring accurate and reproducible results.

### Lateral flow immunoassays

<b>Sample preparation</b>	<b>6</b>
<b>Detection reagent carrier</b>	<b>10</b>
<b>Target capture</b>	<b>12</b>
<b>Sample cleanup</b>	<b>17</b>

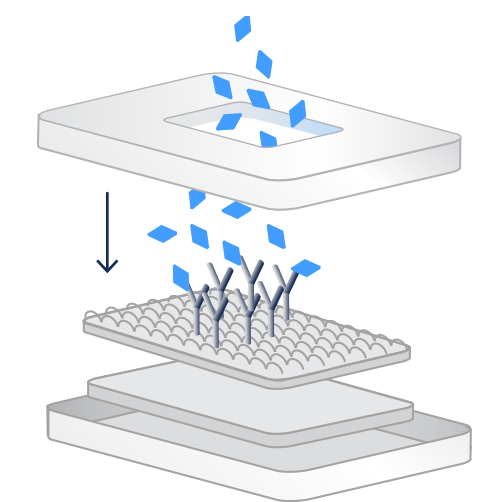
Sample preparation is a strength of lateral flow assays. Sample pads and blood separators can be used to adjust sample properties such as sample pH or mitigating unspecific interactions.



### Flow-through immunoassays

<b>Nitrocellulose membranes</b>	<b>18</b>
<b>Absorbents</b>	<b>19</b>

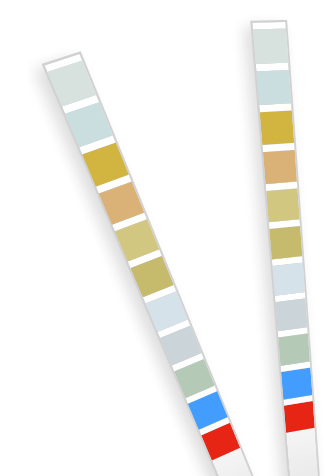
Flow-through assays are generally inexpensive and stable, however they require a reader and a longer time to prepare samples (often via centrifugation) than alternatives.



### Dry chemistry/dipstick assay

<b>Cellulose pads</b>	<b>20</b>
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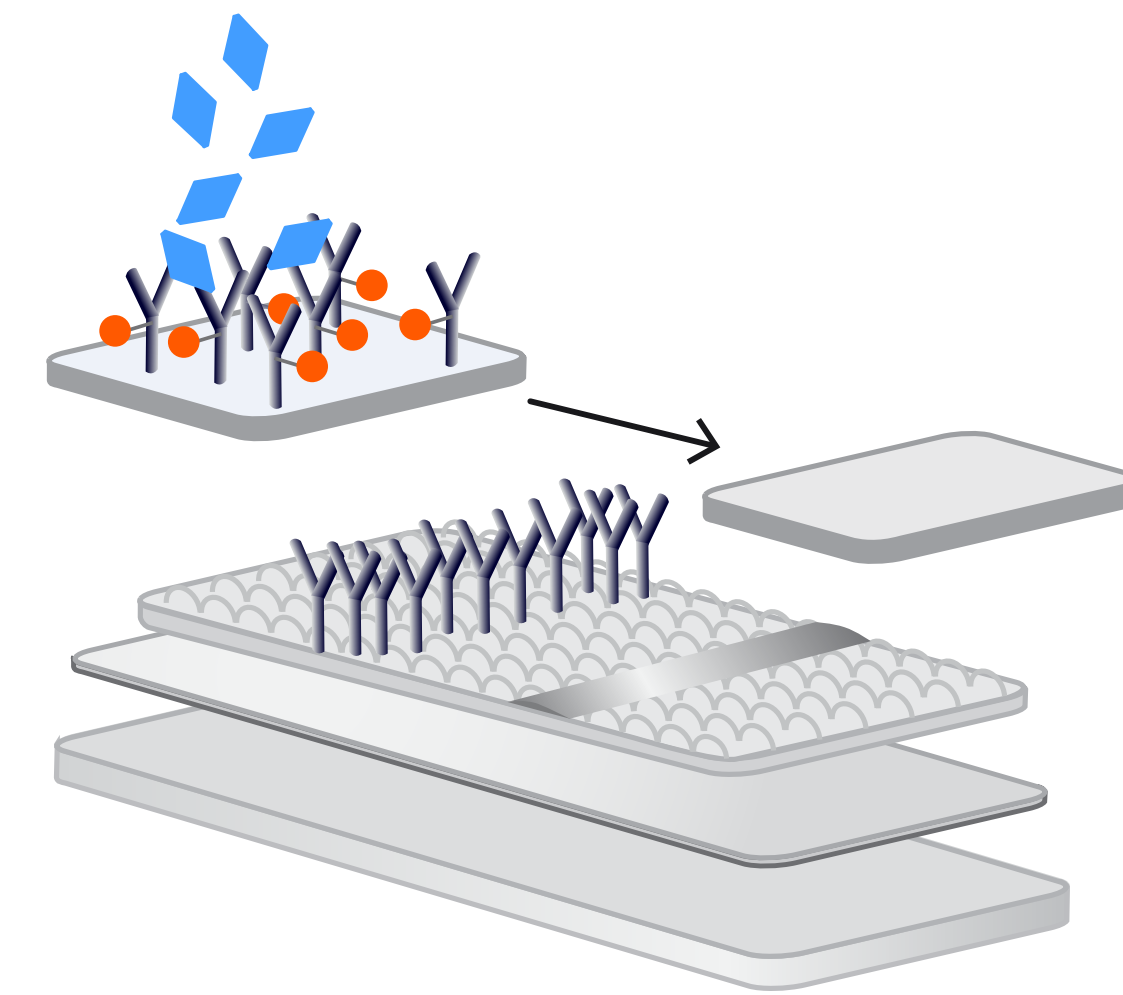
This is a simple to use, easy to read test for determining the presence of low molecular weight analytes via an enzymatic-induced color change.



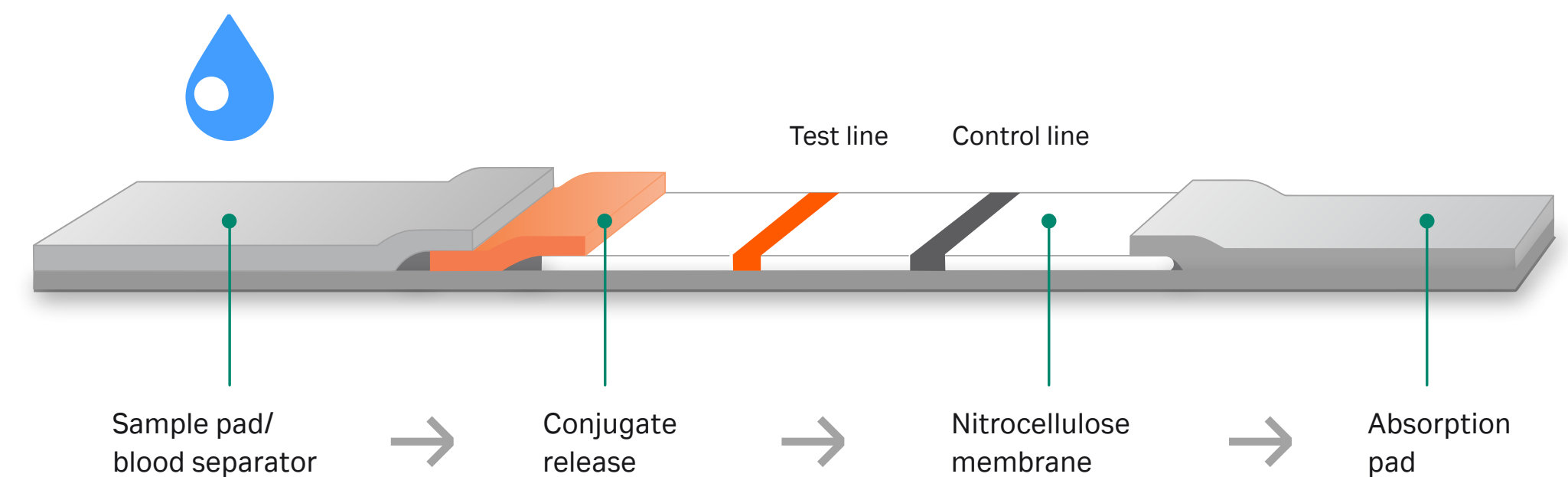
# Lateral flow immunoassays

With a diverse array of products, Cytvia is one of the leading suppliers in lateral flow technology. Our offering includes a wide range of blood separation products, conjugate release pads, nitrocellulose membranes, and absorbents.

Developments in lateral flow immunoassay systems allow for single-step assays that require only the addition of a sample. The sample flows through the device and comes in contact with dried reagents, usually a tagged secondary antibody. The antibody and analyte migrate to a capture zone of membrane-immobilized antibody. Any unreacted tagged antibody flows past the capture zone.



Lateral flow assay.



Drawing of a lateral flow immunoassay, showing its different components

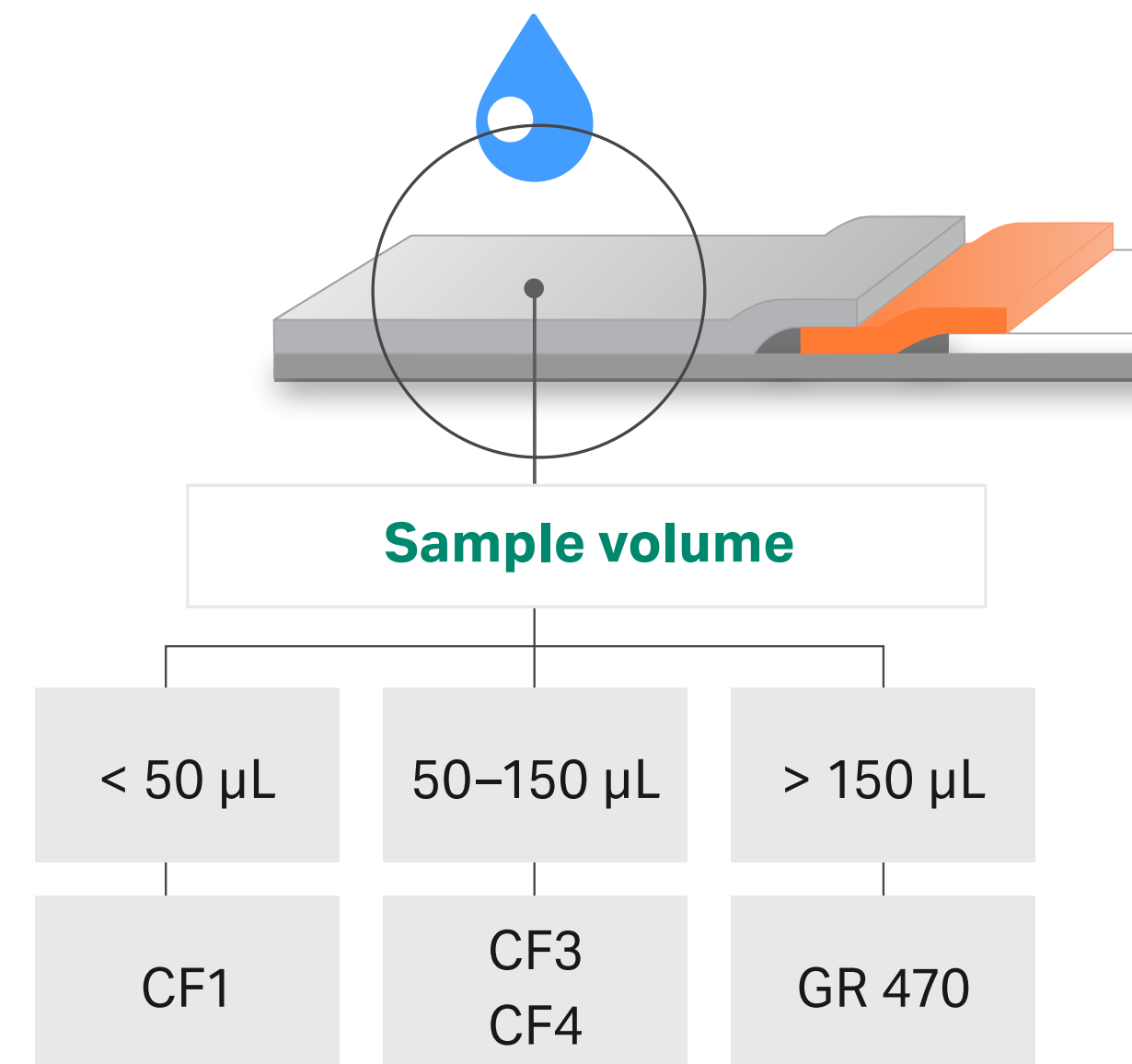
# Sample preparation

## Sample pads for lateral flow immunoassays

Sample pads begin the assay by transporting samples from the point of application to the test components.

### Design considerations when selecting absorption pads

- Pad thickness and absorbency: Generally, the thicker the paper the higher that water absorbency. The overall absorbency determines the total volume of sample that can be applied to the assay.
- Wicking rate: the lateral flow rate of liquid, wicking rate determines how quickly the sample will move through the sample pad and transfer to the conjugate release pad. Ultimately this contributes to the speed of the test.
- Low protein binding: Minimal loss of analyte, so test sensitivity is maintained
- Naturally hydrophilic: Rapid rewetting after prolonged storage
- Minimal leakage along the strip: No contamination of test results



Sample pads selection tree.

# Ordering information

Sample pads for lateral flow immunoassays

Product	Material	Description	Product code	Thickness (µm @ 53kPA)	Wicking rate (s/4 cm)	Water absorption (mg/cm²)
CF1	100% cotton linter	22 mm × 50 m	8111-2250	176	207.3	18.7
CF3	100% cotton linter	22 mm × 50 m	8113-2250	322	174.3	34.6
CF4	100% cotton linter, acid treated to increase wet strength	22 mm × 50 m	8114-2250	482	67.3	49.9
Grade 470	100% cotton linter	22 mm × 50 m	10539995	840	77	78

Slit widths can be customized to meet your needs – please contact your Cytiva representative for more information.



# Blood separators for lateral flow immunoassays

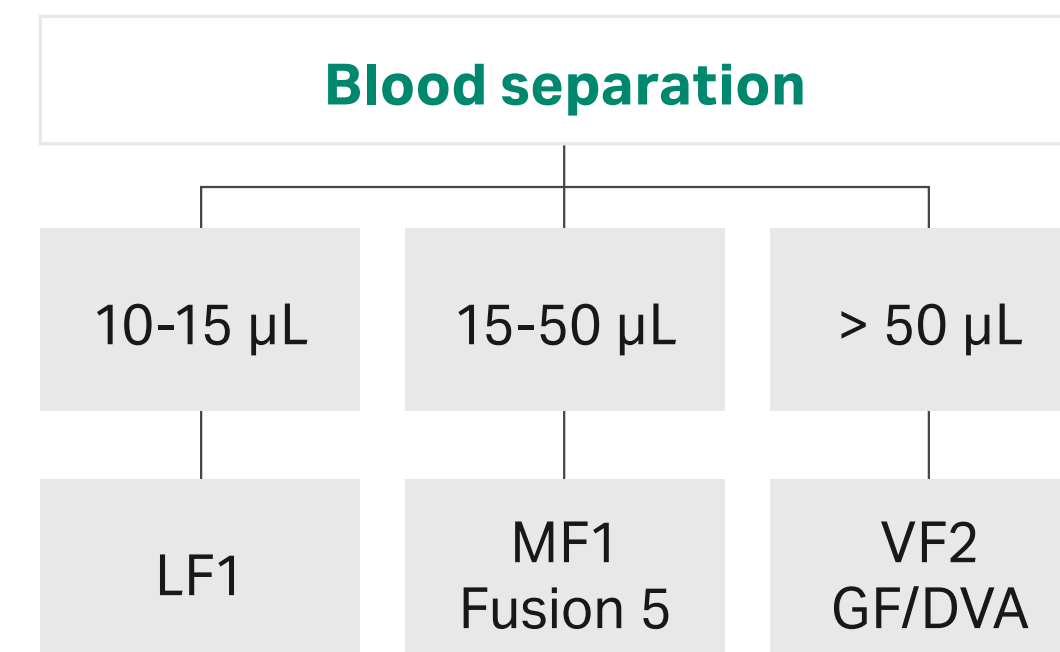
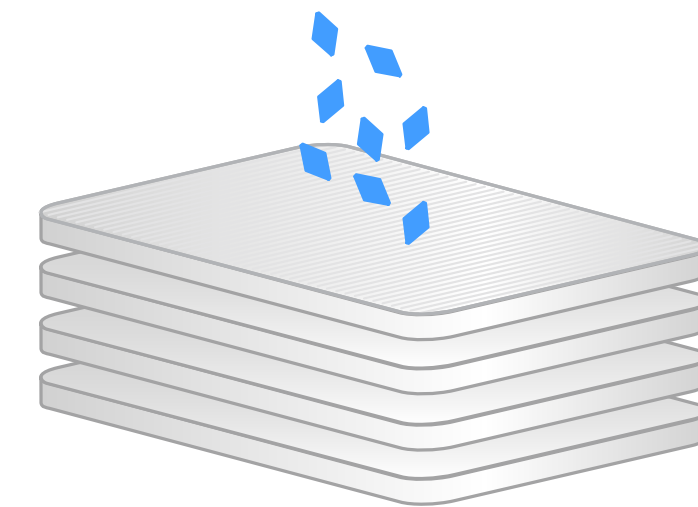
When a test uses blood as the sample matrix, removing red and white blood cells reduces red coloration and eliminates interference from intracellular material, such as DNA.

Diagnostic assays that measure an analyte in blood plasma often use filters for separation, also known as blood separation membranes, most commonly taking advantage of the properties of depth filters.

Key design considerations of pad thickness, water absorption, and wicking rate are similar to the previous section on sample pad considerations.

## Features and benefits

- Separation in 30–120 seconds: Rapid assays save time
- No appreciable red cell hemolysis: Supports reproducible results
- Consistency of materials: Supports assay reliability
- Choice of separation times: Allows for test optimization
- Separators appropriate for a range of blood volumes: Enhances the separation rate according to the volume of sample required



Blood separator selection tree.



# Ordering information

Blood separators for lateral flow immunoassays

Product	Material	Description	Product code	Thickness (µm @ 53kPA)	Wicking rate (s/4 cm)	Water absorption (mg/cm²)
LF1	Bound glass fiber	17 mm × 50 m	8121-1750	247	35.6	25.3
MF1	Bound glass fiber	22 mm × 50 m	8122-2250	367	29.7	39.4
Fusion 5	Bound glass fiber	25 mm × 50 m	8151-9915	370	43.9	42.3
VF2	Bound glass fiber	17 mm × 50 m	8124-1750	785	23.8	86.2
GF/DVA	Bound glass fiber	22 mm × 50 m	8145-2250	785	28.2	93

Slit widths can be customized to meet your needs – please contact your Cytiva representative for more information.

# Detection reagent carrier

## Conjugate release pads for lateral flow immunoassays

Conjugate release pads are critical to lateral flow immunoassays. To ensure consistent performance, the conjugate must dry without damage or aggregation and release rapidly when the sample comes into contact with it.

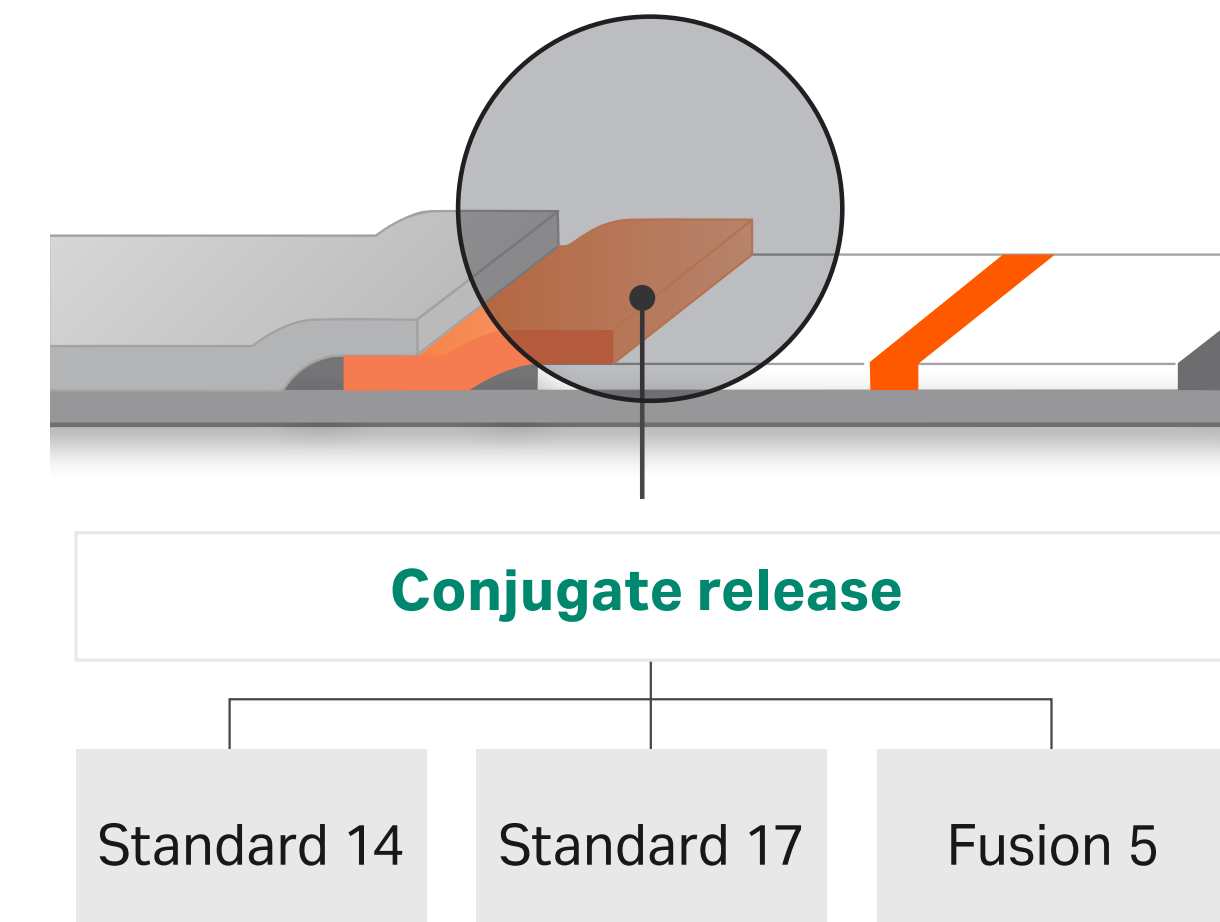
## Design considerations when selecting conjugate release pad

**Water absorption:** As an assay developer considers how many conjugate particles are needed to perform his or her test, water absorption determines the density of conjugates which in turn determines pad size needed to ensure the correct amount of assay dependent conjugate is fed into conjugate release pad.

**Wicking rate:** This determines how fast the mixture of conjugate and sample migrate through the conjugate pad. This in turn affects the release kinetic of the conjugate at the test and control lines. Slower wicking will allow for more interaction time between conjugate and sample but the downside is it also allows more time for unspecific interactions that may occur (and secondarily may make the test slower).

### Features and benefits

- Higher level of conjugate release: Less waste means reduced reagent costs
- Higher capture line intensity, as more conjugate gets to the capture line: Improved sensitivity
- Pad rewets naturally and rapidly every time: Improved consistency



Conjugate release selection tree.  
See table on pg 11 for technical selection criteria.

# Ordering information

Conjugate release pads for lateral flow immunoassays

Grade	Description	Thickness (µm @ 53kPA)	Wicking rate (s/4cm)	Water absorption (mg/cm²)	Percent release of gold conjugate (after 90 s)	Product code
Standard 14	22 mm × 50 m	355	23.1	50.9	75	8133-2250
Standard 17	22 mm × 50 m	370	34.5	44.9	75	8134-2250
Fusion 5	22 mm × 50 m	370	43.9	42.3	>94	8151-9915

Slit widths can be customized to meet your needs – please contact your Cytiva representative for more information.

# Target capture

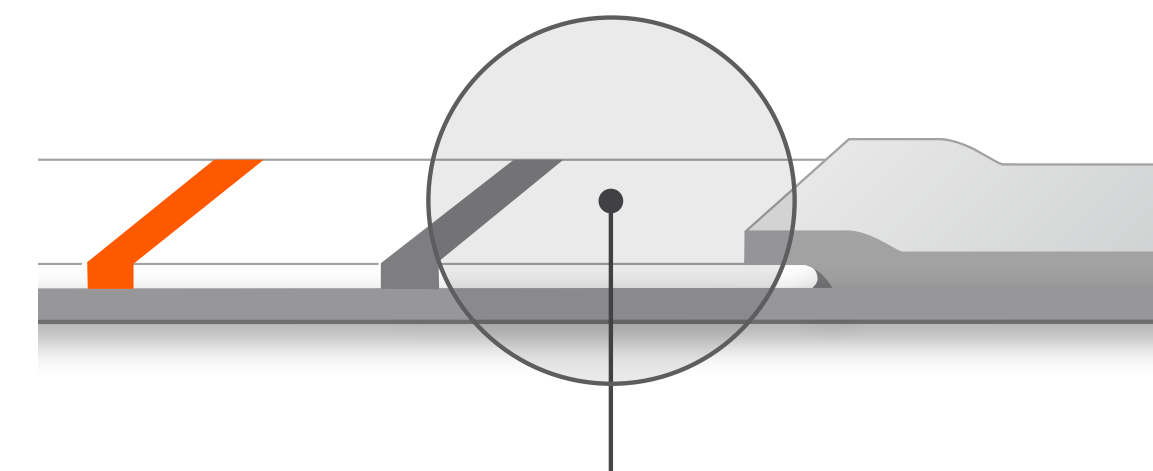
## Faster flow, high performance — backed nitrocellulose membranes

Nitrocellulose membranes from Cytiva provide designers with a range of options to optimize the time that the sample spends at the test and control lines interacting with antibodies which governs the assay's kinetics.

The membrane surface is uniform without any unincorporated nitrocellulose powder and the fine structure fiber distribution provides large internal surfaces for binding proteins.

A carefully designed and rigorous manufacturing process in our ISO 13485:2016 certified facility results in membranes with high reproducibility and very low intra and inter-lot variability.

Our three families of nitrocellulose membrane — FFHP, FFHP Plus, and Immunopore™ — are each treated with different surfactants. Testing a sample from each family is recommended to support optimization of your sample to the best membrane. Generally FFHP Plus grades are recommended for assays using a block on the fly configuration.



**Blood/serum/plasma**

### **Backed membranes**

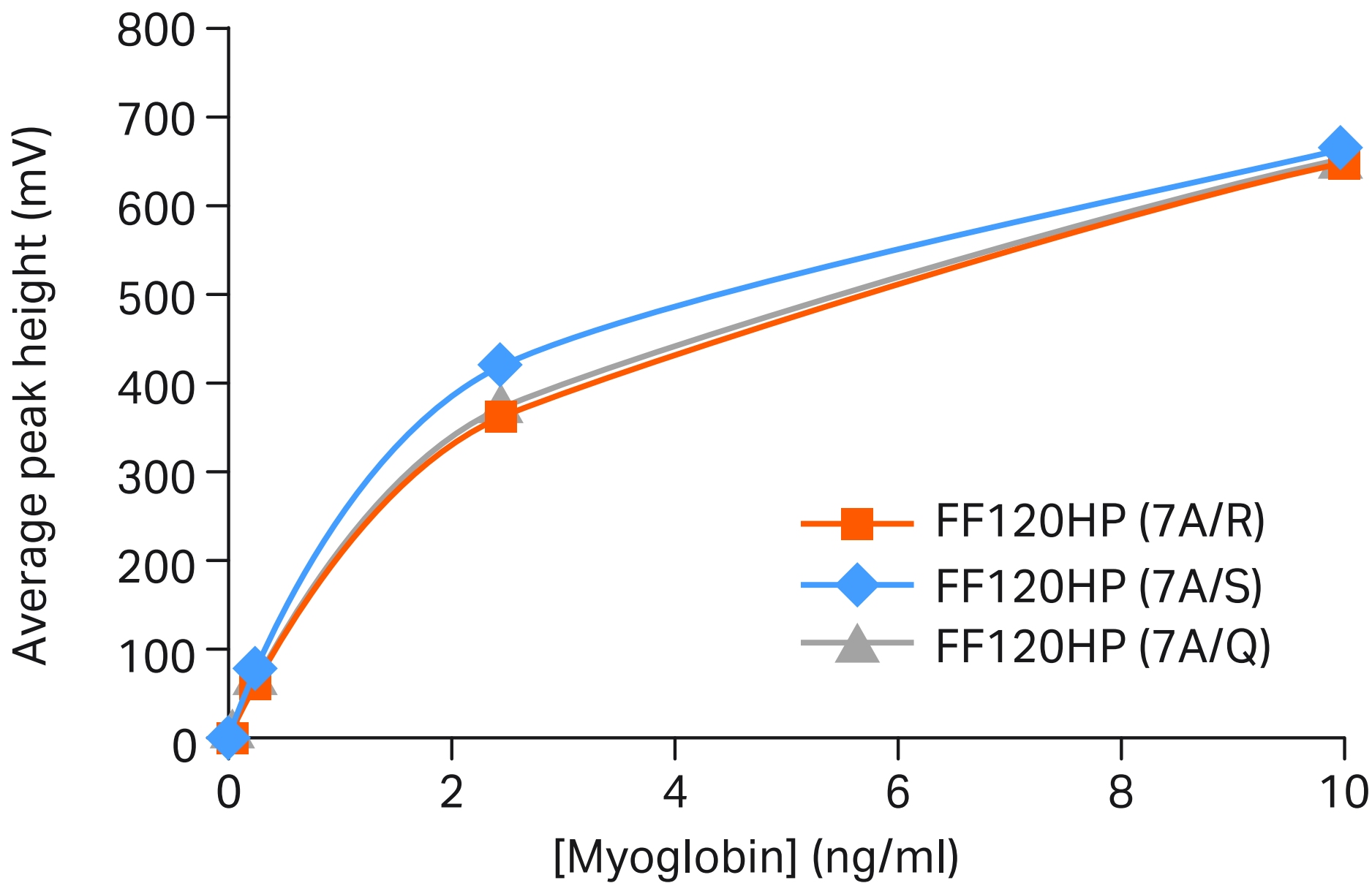
FF80HP  
FF120HP  
FF80HP Plus  
FF120HP Plus  
Immunopore RP

Initial testing should be based on capillary rise time, however many membranes may ultimately need to be tested to determine the best for a given assay given natural variations in the interaction between membrane and sample.

# Lot-to-lot consistency

To ensure consistent assay performance, test designers and manufacturers rely on test materials that perform consistently lot-to-lot. Cytiva products are manufactured to tightly controlled specifications to minimize product variability.

Intra-lot variability of FF120HP based on testing of three rolls from the same casting lot.\*



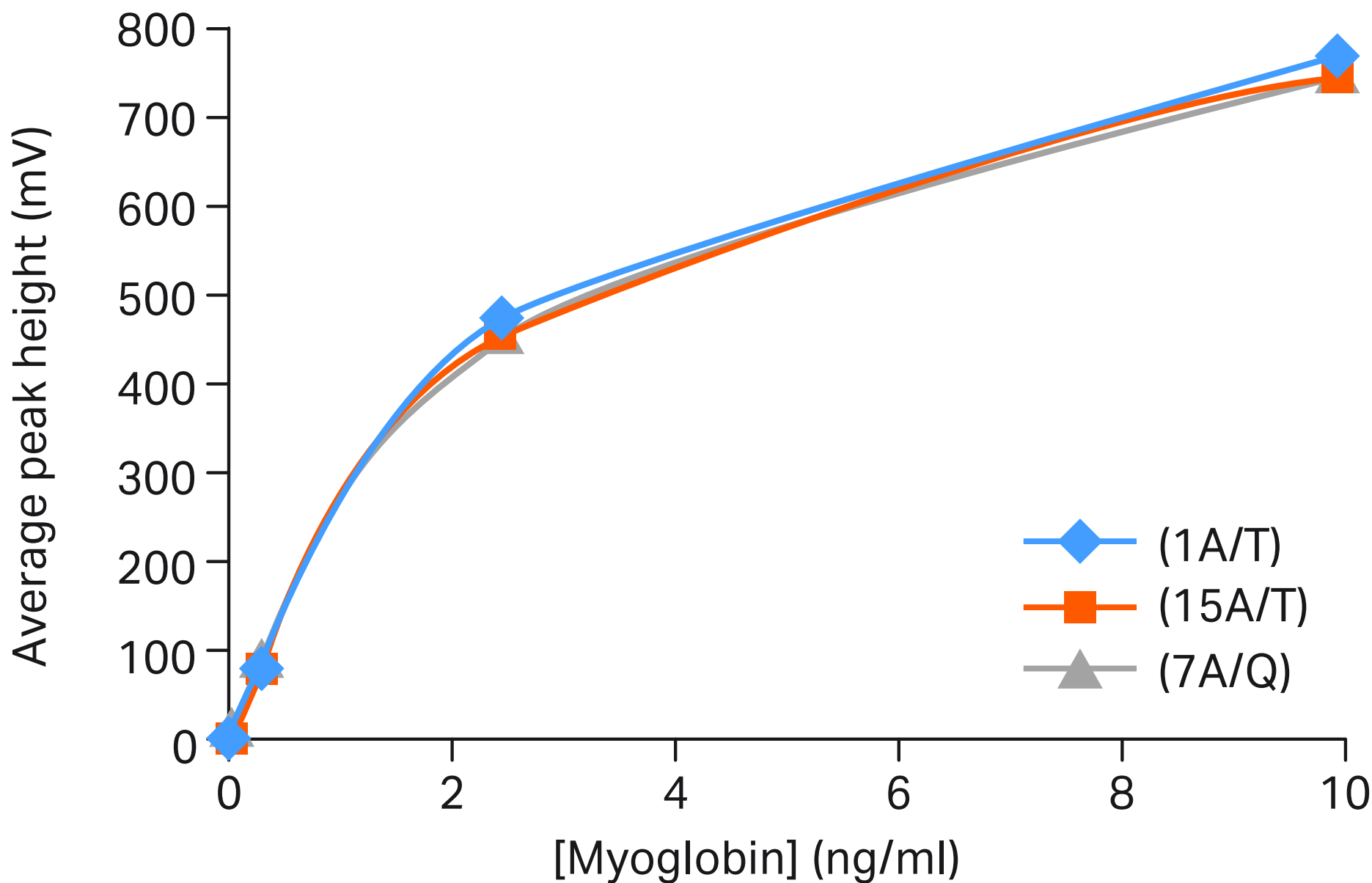
Intra-lot variability of Whatman FF120HP membrane (same casting lot)\*

FF120HP roll number	[Myoglobin] (ng/ml)	Average peak height (mV)	SD	%CV
7A/R	0	0	0	N/A
	0.25	62	10	16.3%
	2.5	367	32	8.7%
	10	656	31	4.7%
7A/S	0	0	0	N/A
	0.25	61	5	8.5%
	2.5	419	23	5.4%
	10	664	29	4.3%
7A/Q	0	0	0	N/A
	0.25	59	13	21.6%
	2.5	380	26	6.7%
	10	657	35	5.3%

\* Results represent 20 replicates per data point.

# Lot-to-lot consistency

Inter-lot variability of FF120HP based on testing of three rolls from three different casting lots.\*



Inter-lot variability of Whatman FF120HP membrane (different casting lots)\*

FF120HP casting lot number	[Myoglobin] (ng/ml)	Average peak height (mV)	SD	%CV
G1471610	0	0	0	N/A
	0.25	75	8	10.4%
	2.5	464	31	6.6%
	10	760	35	4.6%
D013024	0	0	0	N/A
	0.25	68	13	19.2%
	2.5	454	29	6.4%
	10	741	34	4.6%
G1471608†	0	0	0	N/A
	0.25	68	13	19.4%
	2.5	446	41	9.1%
	10	747	36	4.8%

\* Results represent 20 replicates per data point.

† Roll number 7A/Q.

## Membrane blocking

Nitrocellulose has a protein binding capacity which allows for analyte capture and detection at the test line. However, the nitrocellulose that is not on the test line also has the risk of binding the target molecules which could impact the sensitivity of the test. To avoid this, the free nitrocellulose adjacent to the test line and control line must be blocked from binding protein. This can be accomplished in two ways.

1. Blocking in manufacturing – coat the membrane with blocking reagents in a separate manufacturing step after dispensing test and control lines. Non-lateral flow tests always use this method.
2. Block on the fly – blocking reagents integrated in sample pad or conjugate release pad and they migrate in front of the sample. Lateral flow tests may use this method under certain circumstances.

## Membrane backing

A paper backed membrane increases mechanical strength of the membranes, simplifying use in reel-to-reel machines. Direct contact is prevented between the nitrocellulose material and the adhesive from the lamination card where the test elements are mounted. Under almost all circumstances a backed membrane is preferred for lateral flow assays.

## Test speed

Test speed is indicated by capillary rise time. Lateral flow is driven by capillary forces that pull the sample parallel to the membrane surface over ~20–25 mm. This parameter tells how fast monoliquid (water) migrates across the membrane surface. As liquid migrates across test and control lines the kinetics between the capture reagent at these lines and the target molecule determines test sensitivity and specificity. Therefore controlling the time that the sample spends at these lines as impacted by capillary rise time is critical.



# Ordering information

Nitrocellulose membranes

Grade	Description	Capillary rise (s/4cm)	Total caliper (µm)	Product code
FF80HP	20 mm × 50 m 1/pk	60–100	200	10547002
	25 mm × 50 m 1/pk			10547003
	20 mm × 100 m 1/pk			10547121
FF120HP	25 mm × 50 m 1/pk	90–150	200	10547001
	20 mm × 50 m 1/pk			10547006
	210 × 297 mm 10/pk			13549205
FF170HP	20 mm × 50 m 1/pk	140–200	200	10547004
	25 mm × 50 m 1/pk			10547005
	210 × 297 mm 10/pk			13549204
FF80HP Plus	20 mm × 50 m 1/pk	60–100	200	10547041
	25 mm × 50 m 1/pk			10547042
FF120HP Plus	20 mm × 50 m 1/pk	90–150	200	10547039
	25 mm × 50 m 1/pk			10547040
FF170HP Plus	20 mm × 50 m 1/pk	140–200	200	10547043
	25 mm × 50 m 1/pk			10547044
Immunopore RP	25 mm × 50 m 1/pk	90–150	200	78356403

Slit widths can be customized to meet your needs – please contact your Cytiva representative for more information.

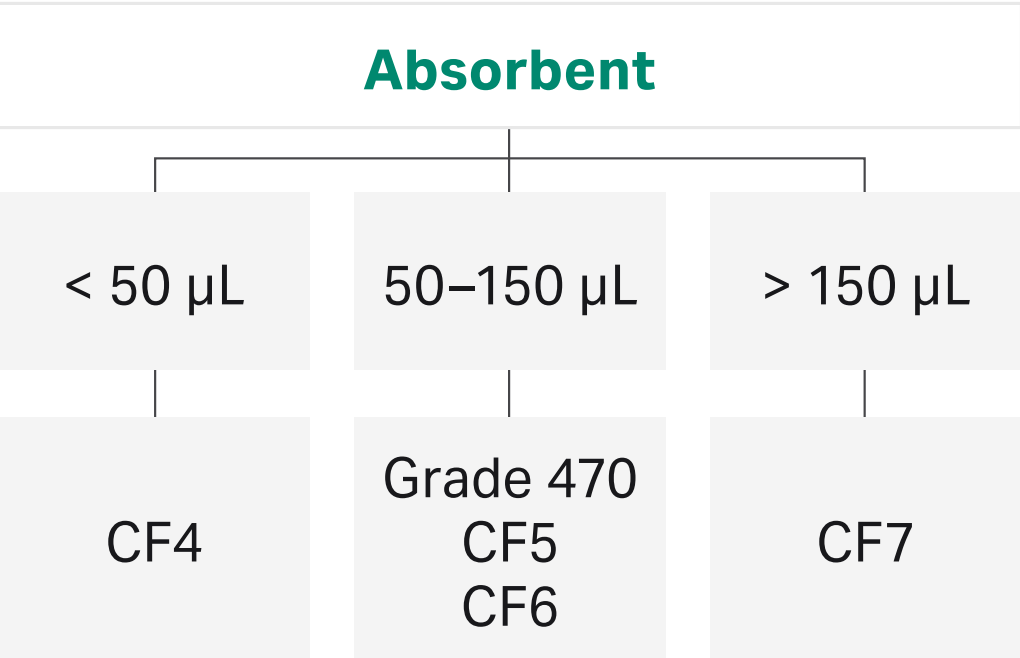
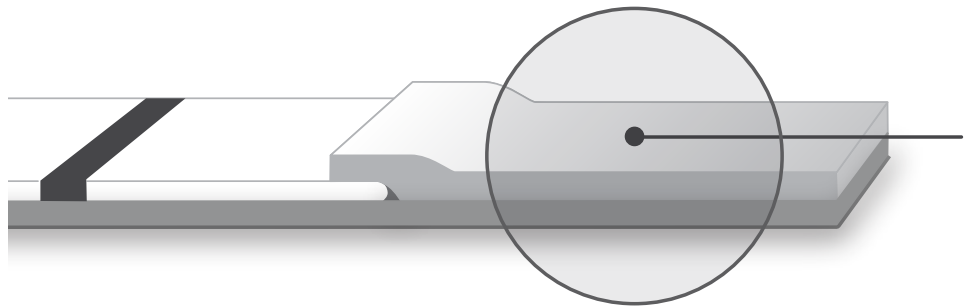
# Sample cleanup

## Absorption pads

Absorption pads at the downstream end of tests control sample flow off of the strip. As such, this affects the amount of time that the sample spends at the test and control lines. Additionally, due to health and safety concerns around unbound sample, choosing an absorbent with sufficient capacity to absorb the full sample volume is an important consideration when designing an immunoassay.

### Features and benefits

- Consistent absorbency: Supports test-to-test reproducibility
- Wide range of thickness, absorbency, and wicking rate: Rapid rewetting after prolonged storage
- Minimal leakage along the strip: No contamination of test results



Absorption pads selection tree.

## Ordering information

Absorption pads

Product	Dimensions	Material	Thickness (µm @ 53kPA)	Wicking rate (s/4 cm)	Water absorption (mg/cm2)	Product code
CF4	22 mm × 50 m	100% cotton linter	482	67.3	49.9	8114-2250
CF5	22 mm × 50 m	100% cotton linter	954	63.3	99.2	8115-2250
CF6	22 mm × 50 m	100% cotton linter	1450	65	136.3	8116-2250
CF7	22 mm × 50 m	100% cotton linter	1750	35	180	8117-2250
Grade 470	460 × 570 mm	100% cotton linter	840	77	78	10318493

Slit widths can be customized to meet your needs – please contact your Cytiva representative for more information.

# Flow-through immunoassays

In a flow-through immunoassay the sample is applied directly to the membrane surface and is allowed to wick through the membrane into an absorbent paper below.

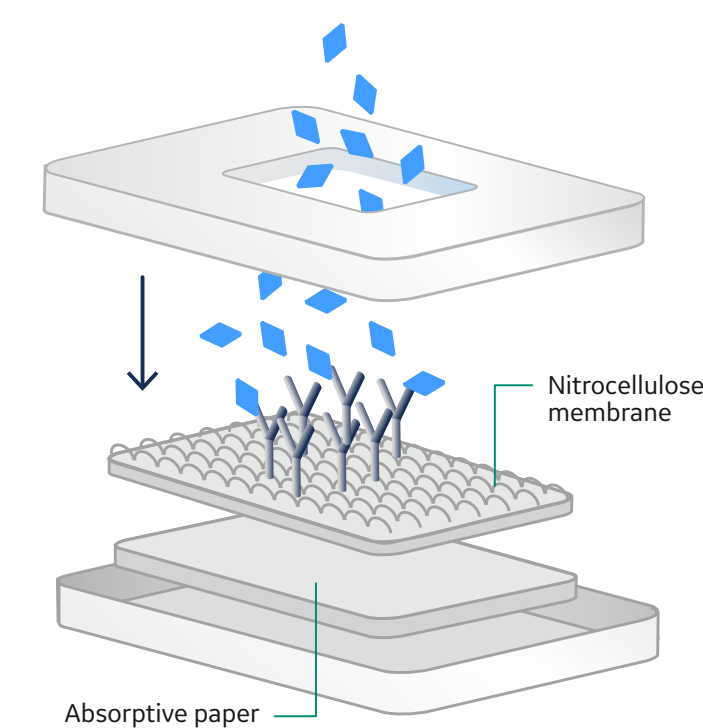
## Nitrocellulose membranes

### Key consideration: pore size

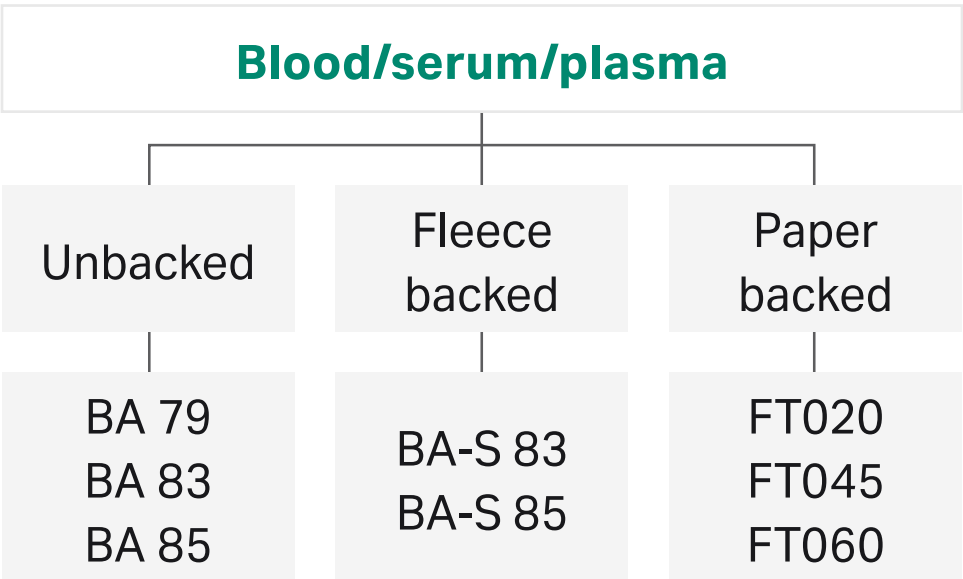
Larger pore size equates to faster test speed but higher speed, in turn, requires faster acting capture reagents. Conversely, smaller pore size equates to higher surface area for conjugates to rest on and therefore, higher protein binding capacity. However, this comes with a cost of slower flow rate.

### Key consideration: membrane backing

Backing adds mechanical support to the membrane while restricting flow rate. Use of an unbacked membrane might be good enough depending on the mechanical load of the sample. If not, fleece or paper backed membranes which are still permeable to the test liquid are options. Paper backed membranes tend to exhibit less bending and curling than fleece backed membranes do which generally makes them preferred for large scale assay manufacturing. However testing of different membrane backings may be appropriate to determine the optimal solution.



Flow-through assay.



Absorption pads selection tree.

## Ordering information

Nitrocellulose membranes

Grade	Dimensions	Pore size (µm)	Thickness (µm @ 53kPA)	Product code
BA 79	300 × 600 mm	0.10	120	10600031
BA 83	300 × 600 mm	0.20	120	10401380
BA 85	300 × 600 mm	0.45	120	10401180
FT020	150 × 150 mm	0.20	~600	10549684
FT045	150 × 150 mm	0.45	~600	10549683
FT060	150 × 150 mm	0.60	~600	10549682

Slit widths can be customized to meet your needs – please contact your Cytiva representative for more information.

# Absorbents

In order to prevent liquid backflow into the test, the absorbent pads used for flow-through assays must be absorbent enough to capture all of the liquid applied to the test (sample, wash buffers, and detection reagents). Assay designers should also ensure that the pad along with other test components can fit comfortably in the test cassette without being compressed in order to get full pad performance.

## Ordering information

Absorbent pads

Grade	Dimensions	Thickness (µm @ 53kPA)	Wicking rate (s/4 cm)	Water absorption (mg/cm <sup>2</sup> )	Product code
CF4	22 mm × 50 m	482	67.3	49.9	8114-2250
CF5	22 mm × 50 m	954	63.3	99.2	8115-2250
CF6	22 mm × 50 m	1450	65	136.3	8116-2250
CF7	22 mm × 50 m	1750	35	180	8117-2250
Grade 470	460 × 570 mm	840	77	78	10318493

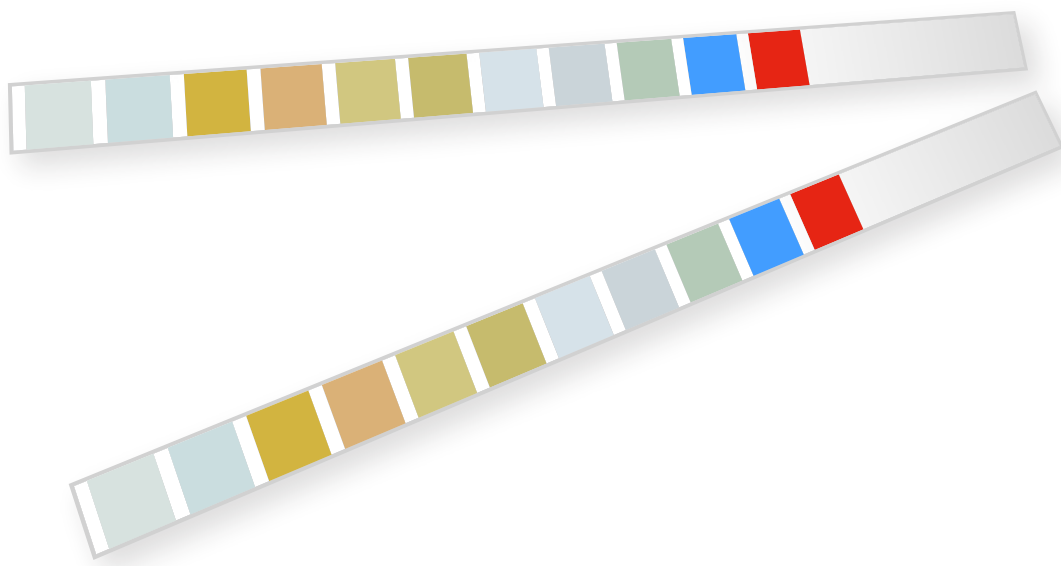
Slit widths can be customized to meet your needs – please contact your Cytiva representative for more information.

# Dry chemistry/ dipstick assay

Dipstick colorimetric assays, in which a cellulose pad is impregnated with a color reagent, are widely used in everything from urine testing to environmental assays. The base cellulose is a key part of the system, and the correct choice of absorbency of the active chemicals required for development of dipstick tests.

## Design considerations when selecting absorbent pads

The most important characteristic is absorption capacity as this will determine whether the pad can handle the sample volume as well as any impregnated reagents. Beyond this material should be tested for compatability with sample liquid and enzymatic reaction products. Pad thickness is also an indicator of mechanical stability which may be important depending on the manufacturing method of the final assay.



Dipstick colorimetric assays.

## Ordering information

Dipstick colorimetric assays

Grade	Thickness (µm @ 53kPA)	Water absorption (mg/cm²)	Dimensions	Product code	Quantity/ pack
CF1	176	18.7	22 mm × 50 m	8111-2250	1
CF2	172	16.1	22 mm × 50 m	8112-2250	1
CF3	322	34.6	22 mm × 50 m	8113-2250	1
CF4	782	49.9	22 mm × 50 m	8114-2250	1
CF7	1750	180	22 mm × 50 m	8117-2250	1
CF10	490	51	100 mm × 50 m	9535669	1

Slit widths can be customized to meet your needs – please contact your Cytiva representative for more information.



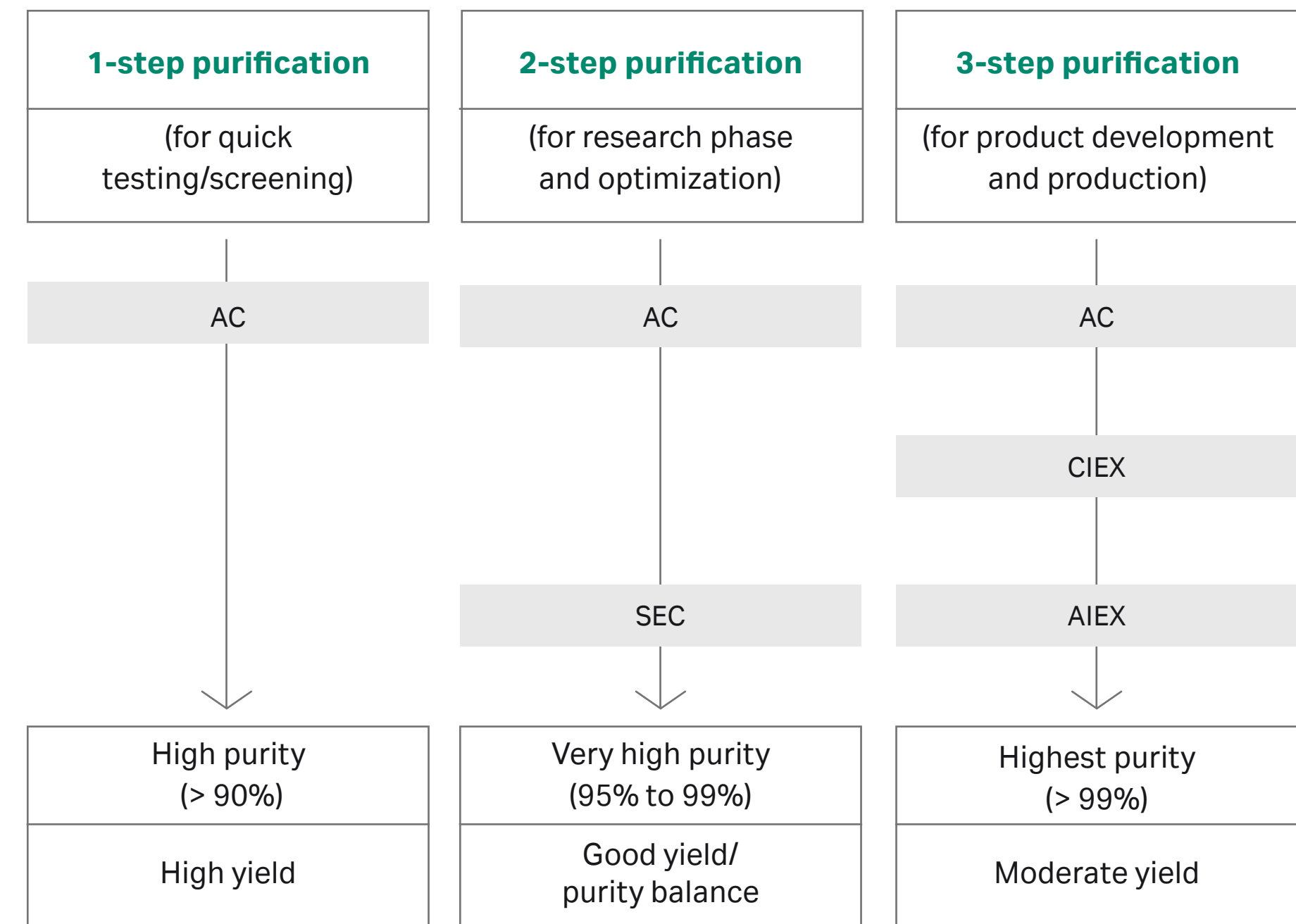
# 02

## Antibody purification

The purity of the capture antibodies used in immunoassay needs to be very high. Often purity around 99% is needed.

In addition to obtaining high antibody purity the security of supply and the quality of the products to produce these assays are important aspects. Cytiva can support with the right tools to give you the pure and active antibody you need for your assay in both research and manufacturing scales.

The figure to the right describes typical proven technique combinations for the purification of antibodies depending on the goal of the purification — high yield, good yield/good purity, or high purity.



1-, 2-, or 3-step protocols may be deployed to purify antibodies depending on the goal of the purification.

AC = affinity chromatography; SEC = size exclusion chromatography; CIEX = cation exchange chromatography; AIEX = anion exchange chromatography

# Cytiva chromatography resins, columns and systems for antibody purification

Please find below a selection of Cytiva lab-scale solutions for purifying antibodies:

## Affinity chromatography columns

- HiTrap™ MabSelect™ Prisma
- HiTrap MabSelect SuRe™
- HiTrap Protein A HP
- HiTrap Protein G HP

## Size exclusion chromatography columns

- Superdex™ 200 Increase
- HiLoad™ Superdex 200 pg
- HiPrep™ Sephacryl™ S-300 HR

## Ion exchange chromatography columns

- HiTrap Capto™ S ImpAct (CIEX)
- HiScreen™ Capto S ImpAct (CIEX)
- HiTrap Capto Q (AIEX)
- HiScreen Capto Q (AIEX)

## ÄKTA™ chromatography systems

- ÄKTA pure
- ÄKTA avant



HiTrap MabSelect Prisma columns



ÄKTA pure



ÄKTA avant

Select the right resins and columns online  
[cytiva.com/purify](https://www.cytiva.com/purify)

Discover Cytiva's ÄKTA systems  
[cytiva.com/AKTA](https://www.cytiva.com/AKTA)



# Which ligand should be used in affinity chromatography?

While protein A and protein G affinity resins are similar in many respects, their specificities for immunoglobulin G (IgG) differ.

Relative binding strengths of antibodies from various species to affinity ligands Protein A, Protein G, and Protein L

Species	Antibody class	Affinity*		
		Protein A	Protein G	Protein L
Human	IgG <sub>1</sub>	+++	+++	+++
	IgG <sub>2</sub>	+++	+++	+++
	IgG <sub>3</sub>	-	+++	+++
	IgG <sub>4</sub>	+++	+++	+++
	IgA	Variable	-	+++
	IgD	-	-	+++
	IgE	-	-	+++
	IgM**	Variable	-	+++
Mouse	IgG <sub>1</sub>	+	+++	+++
	IgG <sub>2a</sub>	+++	+++	+++
	IgG <sub>2b</sub>	+++	+++	+++
	IgG <sub>3</sub>	+	+++	+++
	IgM**	Variable	-	+++
Rat	IgG <sub>1</sub>	-	+	+++
	IgG <sub>2a</sub>	-	+++	+++
	IgG <sub>2b</sub>	-	+	+++
	IgG <sub>2c</sub>	nd	nd	+++
	IgG <sub>3</sub>	+	+	nd
Pig	Total IgG	+++	+++	+++
Dog	Total IgG	+	+	+
Cow	Total IgG	+	+++	-
Goat	Total IgG	-	+	-
Sheep	Total IgG	+/-	+	-
Chicken	Total IgG	nd	nd	-

Species	Antibody class	Affinity*		
		Protein A	Protein G	Protein L
Rabbit	Total IgG	+++	+++	nd
Avian egg yolk	IgY***	-	-	nd
Guinea pig	IgG <sub>1</sub>	+++	+	nd
Hamster	Total IgG	+	+	nd
Horse	Total IgG	+	+++	nd
Koala	Total IgG	-	+	nd
Llama	Total IgG	-	+	nd
Monkey (rhesus)	Total IgG	+++	+++	nd
Other	Kappa light chain (subtypes 1, 3, 4)	nd	nd	+++
	Lambda light chain	nd	nd	-
	Heavy chain	nd	nd	-
	Fab	+/-	+/-	+++
	ScFv	nd	nd	+++
	Dab	nd	nd	+++

+++ = strong binding  
+ = weak binding  
- = no binding  
+/- = weak binding in some cases  
nd = no data available  
\* Protein G and Protein A: Relative binding strengths of antibodies from various species to Protein G and Protein A as measured in a competitive ELISA test. The amount of IgG required to give a 50% inhibition of binding of rabbit IgG conjugated with alkaline phosphatase was determined.  
Protein L: The binding of different radiolabeled IgGs to Protein L-containing *Peptostreptococcus magnus* cells

were measured. Relative binding strength of different IgGs to Protein L is expressed as the percentage of bound IgG to the total amount of IgG. Binding to Protein L occurs only if the immunoglobulin has the appropriate kappa light chains. Stated binding affinity refers only to species and subtypes with appropriate kappa light chains. Data from De Chateau, M. *et al.* On the interaction between Protein L and immunoglobulins of various mammalian species. *Scand.J. Immunol.* **37**, 399–405 (1993).  
\*\* Purified using HiTrap IgM Purification HP columns  
\*\*\* Purified using HiTrap IgY Purification HP columns

# 03

## Design services

### Need to optimize your assay's performance? Cytiva can help.

Assay performance is affected by numerous interactions between your analyte, antibodies, conjugate membrane, sample pad, absorption pad, device housing, etc.

In addition to manufacturing high quality membranes and papers, Cytiva diagnostic experts have over 30 years of experience in assay design.

Let us help you too.

### Live or virtual options available

Choose from three ways to engage. Contact us at [Diagnostics.DesignServices@cytiva.com](mailto:Diagnostics.DesignServices@cytiva.com) or visit us online at [cytiva.com/DiagnosticServices](https://cytiva.com/DiagnosticServices) to request a service.

### Choose from three ways to engage



#### Consultation

Discuss your assay requirements  
and receive relevant samples



#### Design seminar

A Cytiva expert will lead a lecture  
on assay component selection for  
your organization



#### Private workshop

Have a Cytiva expert dedicated  
to optimizing your assay over  
a 3-day deep dive

**cytiva.com/diagnosticservices**

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