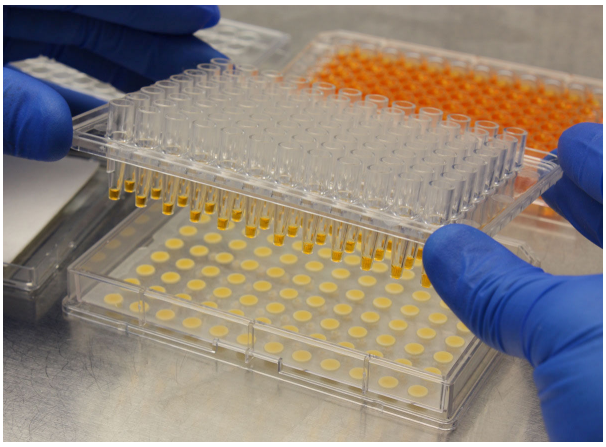
Bel-Blotter™ 96-Well Replicating Tool User Protocol L1010

1. Overview

The Bel-Blotter™ 96-Well Replicating Tool is a sturdy, re-usable polypropylene liquid transfer device comprised of 96 open-ended tips. The replicating tool aspirates approximately 10 µl liquid per tip from 96-well source plates by capillary action, and will deliver 3 - 10 µl, depending upon the replicating medium (plates, filter paper, nitrocellulose, etc.).

The Bel-Blotter 96-Well Replicating Tool distributed by Primorigen Biosciences has been manufactured by Bel-Art Products, and has undergone an additional finishing step to yield perfectly flat and aligned tips optimized for transferring samples to the Spots On Dots™ microarray platform. The replicating tool is ideal for transferring 3 - 6 µl volumes of antibody hybridoma supernatants or other samples to Spots On Dots sheets configured in the 96-Dot format. For transfer of smaller samples to the Spots On Dots 384-Dot format, the PrimaDotter™ 96-pin Transfer Device is recommended (see Section 4: Related Products). For protocols and other information associated with the Spots On Dots technology platform, please visit www.primorigen.com.



Features:

- Compatible with all types of 96-well source plates (round, V, flat-bottom)
- Sturdy, durable polypropylene construction
- Re-usable and economical
- Steam sterilizable
- Finished tips for optimum transfer to nitrocellulose Spots On Dots sheets

2. Components

Bel-Blotter™ 96-Well Replicating Tool (4 pack) **Prod. No. S2067**

Qty	Component	Storage
4 ea.	Bel-Blotter 96-Well Replicating Tool	RT

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3. Protocol

This protocol is specific for applying samples to Spots On Dots™ sheets, 96-Dot format (see Section 4: Related Products) using the Bel-Blotter™ 96-Well Replicating Tool. Please see the appropriate Spots On Dots user protocol for complete procedures.

3.1 Washing and sterilization:

- Have on hand the total number of clean Bel-Blotter replicators needed. We recommend using each Bel-Blotter only once between washes to prevent sample cross-contamination.
- To wash: Submerge Bel-Blotter in a mild detergent solution and soak at least 10 min. Rinse thoroughly under running water, and finish with a dH_2O rinse. Place tip-down on a paper towel to drain remaining water from the tips. Air dry.
- To sterilize: Place a piece of filter paper or paper towel over the tips of the clean replicating tool (prevents discoloration of the tips), wrap in foil, and autoclave.
- The replicator can be sanitized by submersion in 70% alcohol and air-drying. **Do not** flame or expose to strong UV light.

3.2 Applying samples to dry Spots On Dots sheets:

This method is for Spots On Dots sheets that have been printed with proteins, and then blocked in bulk with blocking buffer (10 ml per incubation tray, ≥30 min).

1. Dump Blocking Buffer from incubation tray and use a wadded Kimwipe® or lint-free lab wiper to gently blot the entire surface of the sheet, including the corners of the incubation tray. **Important**: Be careful to remove all visible liquid from between the Dots and from the surface of the Dots. Allow the sheet to air dry for 3-5 min (for damp membranes) or for ≥ 15 min (for dry membranes).

   ➲ *Note: Blocked and dried Spots On Dots sheets can be stored for at least two weeks before use. Allow the membranes to air dry completely (≥ 1 hr) and store in a sealed bag with desiccant at 4 °C.*

2. Place the incubation tray with blocked Spots On Dots sheet on a flat surface.
3. Lower the replicating tool into the 96-well sample source plate, dipping the tips through the liquid meniscus of the samples several times to draw sample into the tips (~3 mm, ~10 µl).
4. Position the loaded replication tool over the Spots On Dots 96 sheet, aligning the tips with the center of the Dots. Carefully lower the replication tool onto the nitrocellulose Dots, and let it rest there.
5. With one hand, gently hold the replication tool in place, and with a fingertip of the other hand, tap across the top of the replicating tool (~15 taps). This step makes sure that sample is delivered to every Dot.

   ➲ *Note: This procedure delivers 3 – 6 µl to each Dot, depending on whether the Dot was damp or dry. A portion of each aspirated sample will remain in the tips of the replicating tool.*

6. Slowly lift the replication tool from the sheet. Visually check that each Dot has received sample. If not, replace the replicating tool onto the sheet, and tap with more gusto.
7. Place the replication tool tip-down on a paper towel to drain remaining samples from the tips. It should be washed before sample residues dry.
8. Incubate the sample-loaded sheet with humidification, as described in the appropriate Spots On Dots user protocol.

3.2 Applying samples to pre-loaded (wet) Spots On Dots sheets:

This method is for Spots On Dots sheets that have been printed with proteins, but not blocked. Blocking buffer is added individually to each Dot, and samples are added without removing the blocking buffer. This approach gives the option of diluting samples as they are added to the Dots (approximately 4-fold) without preparing intermediate dilution plates.

1. Apply 6 µl of blocking buffer to each Dot of a printed Spots On Dots sheet using a multi-channel pipet. Hold the tips above the nitrocellulose Dots and slowly eject, touching the hanging drops to the Dots. Incubate for at least 30 min, with humidification.

   ➲ *Note: Alternatively, blocking buffer can be applied with the Bel-Blotter by following Steps 2-6 of the instructions for applying samples to dry sheets (above). Instead of a sample source plate, substitute a 96-well plate containing blocking buffer (50 – 100 µl/well). Do not use a reservoir, as this aspirates and dispenses too large a volume.*

2. Lower a replicating tool into the 96-well sample source plate, dipping the tips through the liquid meniscus of the samples several times to draw sample into the tips (~3 mm, ~10 µl).

3. Position the loaded replication tool over the Spots On Dots 96 sheet, aligning the tips with the center of the Dots. Carefully lower the replication tool onto the wet nitrocellulose Dots, and let it rest there 5 sec. Tap the top gently 5 times to make sure sample is delivered to every Dot.

   ➲ *Note: This procedure delivers approximately 2 µl sample to each pre-loaded Dot, resulting in a sample dilution of about 4-fold.*

4. Slowly lift the replication tool from the sheet. Place the replication tool tip-down on a paper towel to drain remaining samples from the tips. They should be washed before sample residues dry.

5. Incubate the sample-loaded sheet with humidification, as described in the appropriate Spots On Dots user protocol.

4. Related Products

Kits:	Prod. No.
Spots On Dots™ Antibody Screening Kit (96-Dot format)	S2060-96
Spots On Dots Antibody Screening Kit (384-Dot format)	S2060-384

Hardware:	Prod. No.
PrimaDotter™ 96-pin Transfer Device	S2065
PrimaDotter Alignment Jig	S2066
Bel-Blotter™ 96-Well Replicating Tool (4 pack)	S2067
PrimoriScan™ Flatbed Scanner	S2068

Trademark Information

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