

CentriPure 96-400LD50

Hydrated gel filtration plates, 400µL Well Volume

Sample volume range: 5 to 20µL, Long Drip Directors
Catalog No. CP-0116



Introduction

CentriPure 96-400LD50 gel filtration plates are specifically designed for removing excess terminators and nucleotides from Dye Terminator sequencing reaction mixtures. **CentriPure 96** is precision filled with Zetadex-50, a cross-linked, beaded, composite dextran gel manufactured by emp Biotech. Zetadex-50 is hydrated with sterile, pure water without the use of preservatives, salts or buffers.

The procedure consists of removing the interstitial fluid from the **CentriPure** plate by spinning in a centrifuge equipped to handle deep well plates. The samples are applied to the individual wells and the plate is spun again to collect the purified product in a 96 well plate. Products may be collected into standard 96 well format collection plates (not supplied) for subsequent concentration and denaturing steps.

The **CentriPure** consists of a 96-well filtration plate made from sterile medical-grade polypropylene. Each well has a total volume of 400 µL and a total hydrated gel bed volume of around 320 µL. Each gel bed is supported on an individual ultra high molecular weight PE filter membrane with pore size of 25 microns. The plates are sealed top and bottom to minimize drying.

Materials Provided

- **CentriPure 96-400LD50** Plates, Cat. No. CP-0116

Materials / Equipment Required

- Reusable 96 well wash plates
- 96 well collection plates
- Sealing film (optional)
- Centrifuge with rotor and carriers capable of handling stacked plates (4.1 cm height)
- Multi-channel pipettor and tips

Storage and Stability

The **CentriPure 96-400LD50** plates are stable until the indicated retest date when stored at 2-8°C.

Protocol

Our protocol is at best an approximation of many variables, which include sample variation and pipetting accuracy. Acceleration and deceleration times as well as the precision of the speed of rotation are highly variable among centrifuges. To achieve optimum results, significant deviations from the recommended g-force and centrifuge speeds should be expected.

1. **Important! Allow CentriPure 96 plate to come to room temperature before use.**
2. Remove the adhesive foil from the bottom and then from the top of the **CentriPure 96** plate.
3. Stack the **CentriPure 96** plate on top of a 96 well wash plate and centrifuge at the chosen speed and time (see Table 1).
4. Use an external timer and start timing when the rotor has reached the set speed. Discard the liquid by shaking the wash plate dry. The gel matrix in the wells should appear opaque at this point.
5. Transfer the samples (**20 µL or less**) to the individual wells in the **CentriPure 96-400LD50** plate, taking care to place the samples in the centers of the gel beds.
6. Stack the **CentriPure 96** plate on top of a 96 well collection system and centrifuge at the chosen speed and time. **IMPORTANT! USE THE SAME SPEED AND TIME AS YOU HAVE CHOSEN FOR STEP 3!**
7. Remove the 96 well collection plate containing the cleaned samples and dry in a speed-vac equipped with the appropriate rotor. Alternatively the plate can be sealed for storage.

Centrifugation

Most centrifuges, either bench or floor models, that accept microplate rotors may be used with the **CentriPure 96** protocol. However, the rotor must accept a plate stack approximately 4.1 cm in height (combined height of **CentriPure 96-400LD50** plate and wash plate) as the carrier swings 90° from its horizontal position to the vertical position.

Timing

It is very important to control both the centrifuge speed and the duration of the run. Centrifuges vary by manufacturer in exactly when the internal timers start. Some models begin counting down as soon as the centrifuge run is started so that the ramp up to speed is included in the run time. If the ramp up is slow, the total time at the selected rpm is reduced, thus reducing the total g-force on the plates. We recommend the following procedure:

- Use an external timer to monitor the centrifuge run.
- Start the timer after the rotor has reached the set speed.
- Set the brake on maximum.

As a visual check on the effectiveness of centrifugation, the matrix in the wells should appear opaque and slightly pulled away from the wall after the initial spin prior to sample application. If the matrix appears translucent or shiny, the initial centrifugation conditions are incorrect. Re-spin the plates at the same chosen speed for an additional 2 minutes.

Cushions

Cushions supplied with the centrifuge should be used under the wash plates at all times.

g-force

Speed settings required for each centrifuge to reach the desired g-force will vary with the radius of the rotor used. The centrifuge manufacturer usually supplies a table or nomogram relating rpm to g-force. Alternatively, the following table may be used. Values for fractional radii (i.e., 9.5cm) may be determined by interpolation.

G-Force	Centrifugation Time	RPM required for given rotor radius							
		7 cm	8 cm	9 cm	10 cm	11 cm	12 cm	13 cm	14 cm
850 x g	5 min.	3293	3080	2904	2755	2627	2515	2416	2328
1000 x g	3 min.	3573	3342	3151	2990	2850	2729	2622	2527
1500 x g	2 min.	4375	4093	3860	3660	3490	3342	3211	3094

Table 1. G-force, Centrifugation time, RPM and Rotor radius calculation.

Manual Sample Application

CentriPure 96-400LD50 plates are manufactured using precision filling equipment. This method ensures the extremely uniform gel bed heights required for robotic sample application. Since many users will be loading samples with multi-channel pipettors rather than robots, the following practices should be followed:

- **Samples should be loaded onto the centers of the matrix beds, without touching the pipette tips to the beds.**
- **Allow the sample to “touch-off” onto the gel bed rather than “blowing-out” the pipette tips.**
- **Place the forefinger of your non-pipetting hand alongside the plate row to which the samples are to be applied. Rest the pipette tips on this finger as they are being guided to the center of the gel beds.**

NOTE: This product is intended for research use only.

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