CentriPure™ 96 well
Protocol 800µL plates

Removal of Dye Terminators prior to sequencing:

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 **Gel is already hydrated!**

2 **Important:** Allow plates at least 30 minutes to come to room temperature before use!

Allow refrigerated columns to warm up to room temperature before continuing this procedure!

3 Remove the adhesive foil from the bottom and then the top of the plates.

4 Stack the CentriPure plate on top of a 96-well wash plate and centrifuge at chosen speed and time (see Table 1).

5 Use an external timer and start timing when 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!

6 Transfer the samples (**20µL or less**) to the individual wells in the CentriPure 96 plate, **taking care to place the samples in the centres of the gel beds.**

6 Stack the CentriPure plate on top of a 96-well collection system and centrifuge at chosen speed and time (see Table 1). IMPORTANT: Use the same speed and time as you have chosen for step 3

7 Flow through can be used for sequence analysis or can be stored for later process (after lyophilisation or after sealing with foil).

NOTE: This product is intended for research use only!

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 5.1 cm in height (combined height of CentriPure 96 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 timer starts. 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 before 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 extrapolation.

g-force	Centri-fugation 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	3093

Table 1: g-force, centrifugation time, RPM and rotor radius calculation

Manual Sample Application:

1 **Samples should be loaded onto the centres of the matrix beds, without touching the pipet tips to the beds**

2 **Allow the sample to "touch-off" onto the gel bed rather than "blowing-out" the pipet tips.**

3 **Place the forefinger of your non-pipetting hand alongside the plate row to which the samples are to be applied. Rest the pipet tips on this finger as they are being guided to the centre of the gel beds.**

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