SEP 08, 2020

OPEN ACCESS

DOI:
dx.doi.org/10.17504/protocols.io.7nxhmf

External link:
<https://doi.org/10.1016/j.remle.2020.05.007>

Protocol Citation: Josh Quick 2020. Amplicon clean-up using SPRI beads. **protocols.io**
<https://dx.doi.org/10.17504/protocols.io.7nxhmf>

MANUSCRIPT CITATION:
Fernández-Rodríguez A, Casas I, Culebras E, Morilla E, Cohen MC, Alberola J, COVID-19 and post-mortem microbiological studies. Spanish Journal of Legal Medicine doi: [10.1016/j.remle.2020.05.007](https://doi.org/10.1016/j.remle.2020.05.007)

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Protocol status: Working
We use this protocol and it's working

Created: Sep 25, 2019

Amplicon clean-up using SPRI beads

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MATERIALS

STEP MATERIALS

Elution Buffer (EB) **Qiagen Catalog #19086**

Agencourt AMPure XP **Beckman Coulter Catalog #A63880**

QuantiFluor(R) ONE dsDNA System, 100rxn **Promega Catalog #E4871**

PROTOCOL integer ID:
28087

- 1 Vortex SPRI beads thoroughly to ensure they are well resuspended, the solution should be a homogenous brown colour.

Agencourt AMPure XP **Beckman Coulter Catalog #A63880**

- 2 Add an equal volume (1:1) of SPRI beads to the sample tube and mix gently by either flicking or pipetting. For example add 50 µL SPRI beads to a 50 µL reaction.

- 3 Pulse centrifuge to collect all liquid at the bottom of the tube.

- 4 Incubate for 00:05:00 at room temperature.

- 5 Place on magnetic rack and incubate for 00:02:00 or until the beads have pelleted and the supernatant is completely clear.

- 6 Carefully remove and discard the supernatant, being careful not to touch the bead pellet.

- 7 Add 200 µL of room-temperature 70 % volume ethanol to the pellet.

- 8 Carefully remove and discard ethanol, being careful not to touch the bead pellet.

- 9 go to step #7 and repeat ethanol wash.
- 10 Pulse centrifuge to collect all liquid at the bottom of the tube and carefully remove as much residual ethanol as possible using a P10 pipette.
- 11 With the tube lid open incubate for 00:01:00 or until the pellet loses its shine (if the pellet dries completely it will crack and become difficult to resuspend).
- 12 Resuspend pellet in 30 μL Elution Buffer (EB), mix gently by either flicking or pipetting and incubate for 00:02:00 .
Elution Buffer (EB) **Qiagen Catalog #19086**
- 13 Place on magnet and transfer sample to a clean 1.5mL Eppendorf tube ensuring no beads are transferred into this tube.
- 14 Quantify 1 μL product using the Quantus Fluorometer using the ONE dsDNA assay.
QuantiFluor(R) ONE dsDNA System, 100rxn **Promega Catalog #E4871**

Equipment	
Quantus	NAME
Fluorometer	TYPE
Promega	BRAND
E6150	SKU
https://www.promega.co.uk/products/microplate-readers-fluorometers-luminometers/fluorometers/quantus-fluorometer	LINK