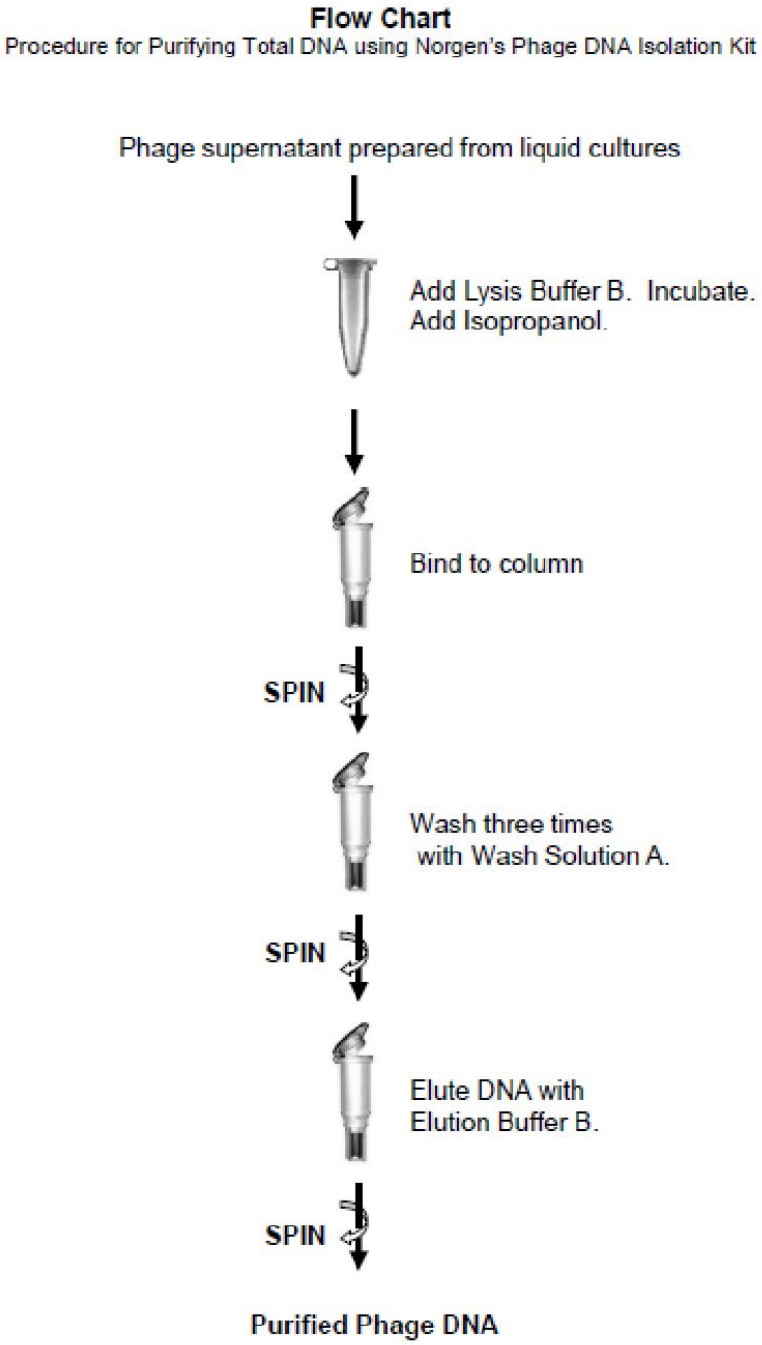
**DNA extraction using the Norgen Phage DNA Kit**

This extraction procedure involves several steps but is far simpler than most other methods. First, DNase is added to the lysates. This will degrade most of the free DNA leftover from the lysed host cells, but the phage DNA will be protected because it is inside the phage capsid. The nuclease is then inactivated by adding EDTA (which chelates divalent cations that are a necessary cofactor for DNase activity).



Read through the protocol first and get yourself familiarized with the steps. Make sure you know where everything you need is located and set any timers in preparation.

1. Label one 2 ml microcentrifuge tube and one 1.5 ml microcentrifuge tube per phage DNA you are extracting with the appropriate phage name.
2. Aliquot 1 ml of DNase treated phage lysate into the 2ml tube.
3. Add 500 µL of **Lysis Buffer B** to the tube**.** Close the cap tightly then vortex on high for 10 seconds.
4. Add 4 µL of 20mg/ml **proteinase K** to the tube then vortex for a few seconds. This step is optional but will help degrade the viral capsids and increase DNA yield.
5. Incubate the reactions at 65°C for 30 minutes. Mix the reaction(s) by gently inverting the tubes 3 times every 10 minutes during the course of this incubation.
6. **Once finished, add 320 µL of isopropanol to the reaction.**
7. Sample Binding to Column
    a. Assemble a spin column with one of the provided collection tubes. Apply up to 650 µL of the lysate to the column and centrifuge for 1 minute at 6,000 x g (~8,000 RPM).
    b. Discard the flowthrough. Reassemble the column and the collection tube. **Note:** Ensure that all of the lysate has passed through into the collection tube. If the entire lysate volume has not passed, centrifuge for an additional 2 minutes.
    c. Repeat step 2a and 2b with remaining lysate from your tube until the entire lysate has passed through the column.
8. Column Wash
    a. Apply 400 µL of **Wash Solution A** (ensure ethanol was added) to the column and centrifuge for 1 minute at 6,000 x g (~8,000 RPM). Discard the flowthrough and reassemble the spin column with its collection tube.
    b. Wash column a second time by adding 400 µL of **Wash Solution A** and centrifuging for 1 minute at 6,000 x g (~8,000 RPM). Discard the flowthrough and reassemble the spin column with its collection tube.
    c. Wash column a third time by adding another 400 µL of **Wash Solution A** and centrifuging for 1 minute at 6,000 x g (~8,000 RPM). Discard the flowthrough and reassemble the spin column with its collection tube.
    d. Spin the column for 2 minutes in order to thoroughly dry the resin at 14,000 x g (~14,000 RPM). Discard the collection tube.
9. DNA Elution
    a. Place the column into your tube labeled “1” from step 1.
    b. Add 75 µL of **nuclease free water** (kept at 65°C in your hot block) to the column.
    c. Centrifuge for 1 minutes at 6,000 x g (~8,000 RPM)
    d. Add a further 25µL of **nuclease free water** to the column.
    e. Centrifuge for 1 minute at 6,000 x g (~8,000 RPM)

Retain the eluate, this is your purified DNA. Purity and DNA concentration can be measured by UV analysis.

**Notes:**

This protocol differs from the manufacturer’s instructions. Refer to the instructions provided with the kit for more information.