# MagVigen™ cfDNA Capture Kit

## Cat # K61003

### Product Contents

- MagVigen™ Plasma DNA Capture Nanoparticles
- Lysis Buffer
- Proteinase K
- Proteinase K buffer
- Wash Buffer 1 Stock
- Elution Buffer

### Materials Needed

- Isopropanol
- Ethanol
- SDS, 20%
- Magnetic Rack (NVIGEN Cat# A20006)

### Note:

The MagVigen™ Plasma DNA Capture Kit (K61003) is capable of capturing small circulating cell-free DNA (>30bp) from plasma and serum.

### Protocols

1. Prepare the plasma samples: if using frozen plasma, thaw under room temperature. Centrifuge plasma at 12000x g for 5 mins to remove any blood and cell debris.
2. Prepare Lysis Buffer: Heat at $60^0$C for a few minutes if there is solid to make clear solution.
3. Prepare Proteinase Solution: Add Proteinase K Buffer to Proteinase K powder vial. Vortexing to mix well. Store at $-20^0$C.
4. Prepare Wash Buffer 1: Calculate needed quantity according to Table 1. Add 450 ul Isopropanol to every 550 ul of Wash Buffer 1 stock. Fresh make the buffer each time.
5. Check Table 1 on the total volume to decide types of centrifuge tubes (1.5ml, 2ml, 5ml, 15ml or 50ml) to use.
6. Add proteinase K solution to the bottom of a sample tube, add plasma to the tube. Vortexing for 5s to mix well, briefly spin down.
7. Add 20% SDS to the tube. Vortexing for 5s to mix well, briefly spin down.
8. Add Lysis Buffer to the tube. Vortexing for 1 min to mix well. Briefly spin down. Incubate at 60°C for 30 min.
9. Add MagVigen™ Plasma DNA Capture nanoparticles. Slightly shake the vial to disperse. Then add Isopropanol, vortexing to mix well, briefly spin down, and keep vortexing for 45 min.
10. Put the reaction tube on a magnetic rack to pellet the beads until the solution is clear (10-20 minutes depending on the strength of the magnet and the sample volume).
11. Slowly remove the supernatant. Be careful not to take any beads, remove as much solution as possible. Tap the magnetic rack on a solid surface to allow residual solution to settle down to tube bottom, remove all supernatant.
12. Take tube off magnet, add Wash Buffer 1. Mix by pipetting or vortexing. Transfer the solution to a 1.5 or 2 ml tube if starting in larger 15 or 50 ml tubes. Briefly spin down. Pellet the beads on the magnetic rack(~ 10 minutes), remove the supernatant. Tap the magnetic rack on a surface. Remove all supernatant.
13. Wash the beads pellet using 75% Ethanol. Do not need to fully disperse the bead pellet. The tubes can remain in position by the magnetic rack. Slightly tilt forward and backward of the magnetic rack with the sample tubes in. Then briefly spin down so that no solution remains in the cap. Pellet the beads on a magnetic rack (~3 min) and remove all supernatant. Tap the magnetic rack on a solid surface to settle supernatant residue to the bottom of the tube. Remove all supernatant.
14. Wash the beads pellet again using 75% Ethanol. Pellet the beads (~ 2-3 min) and remove all supernatant.
15. Leave tubes on the magnetic rack and air dry the pellet to evaporate all ethanol. This takes 2-3 minutes.
16. Add desired amount of Elution Buffer to the beads, pipette up and down until all pellet has re-dispersed completely. Keep beads dispersed in elution buffer for 3 minutes.
17. Briefly spin down. Then set the tube on a magnetic stand for 2-3 minutes.
18. Collect the supernatant without disturbing the magnetic beads pellet. The supernatant contains extracted DNA. Put the tube by the magnet when pipetting the DNA solution out for downstream experiments. The extracted cfDNA solution can be stored at $-20^0$C if not immediately in use.

**Table 1. Reagent volume used in each step.**

| Reagent | ul | ul | ul | ul | ul | ul |
|---|---|---|---|---|---|---|
| Proteinase K | 3 | 7.5 | 15 | 30 | 45 | 60 |
| **Plasma** | **200** | **500** | **1000** | **2000** | **3000** | **4000** |
| SDS (20%) | 10 | 25 | 50 | 100 | 150 | 200 |
| Lysis Buffer | 95 | 237.5 | 475 | 950 | 1425 | 1900 |
| Magnetic Beads | 14 | 35 | 70 | 140 | 210 | 280 |
| Isopropanol | 240 | 600 | 1200 | 2400 | 3600 | 4800 |
| Wash Buffer 1 | 80 | 200 | 400 | 800 | 1200 | 1400 |
| Wash 2 / 3 (75% Ethanol) | 80 | 200 | 400 | 800 | 1200 | 1400 |
| | 80 | 200 | 400 | 800 | 1200 | 1400 |
| Elution Example | 10 | 15 | 25 | 40 | 45 | 50 |
| Total Volume | 0.6 ml | 1.4 ml | 2.8 ml | 5.6 ml | 8.4 ml | 11.2 ml |