

## MagPure Forensic DNA Kit

### Introduction

This product is specially designed for DNA extraction from bone, hair, fingernail, seminal strain, ect forensic samples. Especially designed for **forensic detection**. The obtained DNA can be directly used for PCR, STR detection and other down stream applications.

### Bottle Reagent Kit Contents

Product	D635900D	D635901D	D635902D	D635903D
Preps per Kit	20 Preps	48 Preps	96 Preps	480 Preps
MagPure Particles N	1.1 ml	1.1 ml	2.2 ml	11 ml
Buffer ATL	20 ml	40 ml	80 ml	2 x 200 ml
Buffer BGL	20 ml	40 ml	80 ml	2 x 200 ml
Buffer BST1	30 ml	60 ml	120 ml	550 ml
Buffer GW2*	10 ml	20 ml	25 ml	2 x 50 ml
Proteinase K	24 mg	50 mg	120 mg	2 x 220 mg
Protease Dissolve Buffer	1.8 ml	5 ml	10 ml	30 ml
DTT	235 mg	235 mg	235 mg	3 x 235 mg
Elution Buffer	5 ml	10 ml	10 ml	30 ml

## Prefilled Kit Contents

- Precast reagent, V bottom plate

Product		Contents and volume	D6359D-TL-06	D6359D-S-48
Buffer BGL			80 ml	40 ml
Buffer ATL			80 ml	40 ml
Proteinase K			120 mg	50 mg
Protease Dissolve Buffer			10 ml	5 ml
DTT Powder			2 x 235 mg	235 mg
Elution Buffer			5 ml	5 ml
TL-Tip			12pcs	24pcs
Tip bottom plate/ Tip base reagent strip	Row 1/7: empty		6 plates	48 strips
	Row 2/8: 500µl Buffer BST1			
	Row 3/9: 500µl Buffer BST1			
	Row 4/10: 20µl MagPure Particles N			
	500µl Buffer GW2			
	Row 5/11: 500µl Buffer GW2			
	Row 6/12: 60µl Elution Buffer			

## Storage and stability

Proteinase K should be stored at 2–8°C upon arrival. However, short-term storage (up to 12 weeks) at room temperature (15–25°C) does not affect their performance. DTT Powder is easily oxidized, should be shipped at -20~8°C and stored at -20°C upon arrival, keep dry. The remaining kit components can be stored at room temperature (15–25°C) and are stable for at least 18 months under these condition.

## Preparation

- Add 1.2ml (20 Preps) or 2.4ml (48 Preps), 6.0ml (96 Preps) or 2x1 ml (480 Preps) Protease Dissolve Buffer to the bottle of Proteinase K and store at -20~8°C after dissolve.
- Add 1.5ml Elution Buffer to each bottle of DTT dry powder, vortex to mix thoroughly. Use or store at -20°C. Do not freeze and thaw repeatedly.
- Add 40ml (20 Preps) or 80ml (48 Preps), 100ml (96 Preps) or 2x200ml (480 Preps) absolute ethanol to the bottle of Buffer GW2 and store at room temperature.

## Bone grinding

The quality of STR maps from bone samples depends on the type of bone, age, and environmental storage conditions. Soil conditions and moisture have a profound effect on DNA quality. The success of the extraction process depends on the degree of grinding, which can be achieved by physical grinding or with a bit operated at a low speed to reduce heat build-up. The extraction process works best for fine-ground bone meal, where cells scattered throughout the bone matrix are easier to digest.

**Bone meal grinder:** Pre-cool teeth or bones with liquid nitrogen, and pre-cool bone meal grinders with liquid nitrogen. Transfer the sample to the bone meal grinder, beat it hardly with a hammer several times, pre-cool the grinder with liquid nitrogen, beat it several times until the sample forms a partial fine powder and small bone fragments, transfer the sample to the container, gently shake, and continue grinding the large sample into powder. Gently oscillate in the container to pick out the fine powder for extraction process.

**Bead mill:** Please refer to the protocol of bead mill.

## Protocol 1. single tube operation

1. **Bone/Teeth:** Transfer 100~150mg bone meal into a new 2.0ml centrifuge tube. Add 400µl Buffer BGL, 4µl 1M DTT, and 40µl Proteinase K, inverting several times. Mix by shaking at 1000-1500rpm at 55°C water bath for 3~24 hours.

**Other forensic materials:** Transfer samples such as hair, fingernail, seminal strain, tissue, ect into a new 2.0ml centrifuge tube. Add 400µl Buffer ATL, 4µl 1M DTT, and 40µl Proteinase K, inverting several times. Mix by shaking at 1000-1500rpm at 55°C water bath for 3~24 hours.

Note : The digestion of small bone powder for 3 hours can also obtain enough DNA. If time available, it can extend the digestion time to 24 hours.

2. Centrifuge at 13,000 x g for 3 minutes to remove undigested sediments.
3. **Transfer 250~300µl supernatant to a new centrifuge tube. Add 20µl MagPure Particles N and 500µl Buffer BST1, mix thoroughly by inverting for 10~15 times.** Stay at room temperature for 3 minutes with occasionally inverting to mix. Place the tube to the magnetic stand for 2 minutes until the beads have form a tight pellet. Then remove the supernatant.
4. **Add 500µl Buffer BST1 and vortex for 10 seconds.** Place the tube to the magnetic stand for 2 minutes until the beads have form a tight pellet. Then remove the supernatant.
5. Add 500µl Buffer GW2 and vortex for 10 seconds. Place the tube to the magnetic stand for 2 minutes until the beads have form a tight pellet. Then remove the supernatant.
6. Repeat step 5 once
7. Centrifuge shortly to collect liquid on the tube. Place the tube to the magnetic stand and remove all the liquid carefully. Open the lid and air dry for 10 minutes..
8. Add 50µl Elution Buffer to the sample, re-suspend the beads by vortex. Incubate at 55°C for 10 minutes with shaking. If there is no shaking device, vortex 2~3 times to mix.
9. Place the tube to the magnetic stand for 3 minutes. Transfer the supernatant containing purified DNA to a clean 1.5ml centrifuge tube.

## Protocol 2: 32/48 channel nuclear acid extraction machine.

1. **Single well sample (150mg bone or teeth)** : transfer 100~150mg bone meal into a new 2.0ml centrifuge tube, add 400µl Buffer BGL, 4µl 1M DTT and 40µl Proteinase K, inverting several times. Mix by shaking at 1000-1500rpm at 55°C water bath for 3~24 hours.

**Double well sample (300mg bone or teeth)** : transfer 150~300mg bone meal into a new 2.0ml centrifuge tube, add 700µl Buffer BGL, 7µl 1M DTT and 40µl Proteinase K, inverting several times. Mix by shaking at 1000-1500rpm at 55°C water bath for 3~24 hours.

**Single well sample (other forensic samples)**: Transfer samples such as hair, fingernail, seminal strain, tissue, ect into a new 2.0ml centrifuge tube. Add 400µl Buffer ATL, 4µl 1M DTT, and 40µl Proteinase K, inverting several times. Mix by shaking at 1000-1500rpm at 55°C water bath for 3~24 hours..

**Double well sample (other forensic samples)**: Transfer samples such as hair, fingernail, seminal strain, tissue, ect into a new 2.0ml centrifuge tube. Add 700µl Buffer ATL, 7µl 1M DTT, and 40µl Proteinase K, inverting several times. Mix by shaking at 1000-1500rpm at 55°C water bath for 3~24 hours.

2. Centrifuge at room temperature at 13,000 x g for 5 minutes to remove undigested impurities.
3. **Bottled reagent kit**: add the reagents to the 96 well plate following the above table of prefilled kit contents.

**Prefilled kit**: invert the 96 well plate to suspend the magnetic beads completely. Pat the plate to make reagents fall back to the bottom of plate. Stay the plate at table for 1 minute, remove the sealing pack and sealing film. .

4. **Single well sample**: Add 250~300µl supernatant to the hole of row 2 and 8.  
**Double well sample**: Divide the sample into two same parts, add 250~300µl supernatant to the hole of row 2,8,3 and 9.
5. Insert the magnetic tip (TL-Tip) and 96-well plate in to the machine (hole A1 is placed at the left inner corner). Turn on the machine and start the program.
6. After the extraction complete, ~ 30 minutes, the extraction is complete. Remove the 96-well

plate and magnetic tip.

7. Transfer the purified DNA into a new 1.5ml centrifuge tube and store at  $-20\sim 8^{\circ}\text{C}$ .

**【Mag Pure 32 Extractor program recommendation】**

NO	Step	well	volume	Mix time		waiting		Magnetic time			Magnetic absorption	heating	
				time	speed	time	place	lift	Fluid level	bottom		plate	temperature
1	Magnetic absorption	4	500	30s	7	0	0	60s	0	0	Auto	/	/
2	Binding 1	2	800	240s	7	0	0	60s	15	15	Auto	/	/
3	Wash 1	3	800	240s	7	0	0	60s	15	15	Auto	/	/
4	Wash 1	4	500	60s	8	0	0	60s	0	0	Auto	/	/
5	Wash 2	5	500	60s	8	0	0	60s	0	0	Auto	/	/
6	Dry	6	500	0	8	3min	0	0	0	0	Auto	/	/
7	Elution	6	100	240s	8	0	0	60s	0	50	Auto	/	/
8	Discard magnet	5	500	30s	8	0	0	0	0	0	Auto	/	/