

XpressDNA Plasmid Kit

Protocol for isolation of highly pure plasmid DNA from bacterial samples.

Process Flow



Kit Contents

| Components | Storage Conditions | Shipping Conditions |
|---------------------------|--------------------|---------------------|
| Plasmid Extraction Buffer | 2 - 8°C | RT |
| Plasmid MagNa Mix | 2 - 8°C | RT |
| Plasmid Wash Buffer | 2 - 8°C | RT |
| Plasmid Elution Buffer | 2 - 8°C | RT |
| MagNa Stand (optional) | RT | RT |

^{*} RT denotes 15 - 25°C.

Materials not provided with the kit

- 1. 100% Ethanol to Wash Buffers as indicated on the bottle.
- 2. Water bath/heat block at 80°C.

Important

Pay attention to standard lab practices and safety information before beginning the procedure. For more information, refer the appropriate Material Safety Data Sheet (MSDS) available from the product supplier or download from our website http://www.maggenome.com/

Technical Support

For any product related queries please write to us on info@maggenome.com, sales@maggenome.com, support@maggenome.com, support@maggenome.com,

MG18P1-50/250



Protocol

| 1. Harvest 1.5 ml of overnight bacterial culture (OD ₆₀₀ >1 - 1.5) in a 1.5 ml tube and centrifuge at 10,000 rpm for 5 minutes. Carefully discard the supernatant and remove traces of excess medium, if any. (Note: For low copy number plasmids, bacterial culture volume can be varied up to 2 ml) 2. Add 150 μl of Plasmid Extraction Buffer and gently resuspend the pellet by pipette mixing, until the cells are completely dispersed in the buffer. 3. Incubate at 80°C for 3 minutes. 4. After incubation, centrifuge the lysate at 14,500 rpm for 10 minutes. 5. Transfer the supernatant to a fresh 1.5 ml tube without disturbing the pellet. (Note: Vortex the MagNa Mix thoroughly before the next step) 6. Add 120 μl of Plasmid MagNa Mix to the supernatant and invert mix gently 10 - 12 times. Do not vortex. 7. Incubate at RT for 5 minutes. 8. After incubation, place the tube on a MagNa Stand for 2 minutes or until the solution becomes clear. 9. Discard the supernatant without removing the tube from the MagNa Stand. 10. To the magnetic nanoparticles, add 200 μl of Plasmid Wash Buffer and invert mix gently 5 - 6 times without removing the tube from the MagNa Stand. 11. Discard the supernatant without removing the tube from the MagNa Stand. 12. Repeat steps 10 - 11. 13. Air dry the MNPs the tube on the MagNa Stand at RT for 5 - 10 minutes. | Bacterial Lysate Preparation 2. Add 150 pipette 1 3. Incubate 4. After inc 5. Transfer (Note: Vo 6. Add 120 times. D 7. Incubate | crifuge at 10,000 rpm for 5 minutes. Carefully discard the supernatant ove traces of excess medium, if any. In low copy number plasmids, bacterial culture volume can be varied up to 2 ml) In plasmid Extraction Buffer and gently resuspend the pellet by mixing, until the cells are completely dispersed in the buffer. The at 80°C for 3 minutes. The supernatant to a fresh 1.5 ml tube without disturbing the pellet. The supernatant to a fresh 1.5 ml tube without disturbing the pellet. The supernatant MagNa Mix thoroughly before the next step) Oull of Plasmid MagNa Mix to the supernatant and invert mix gently 10 - 12 |
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| | 13. Air dry | the MNPs the tube on the MagNa Stand at RT for 5 - 10 minutes. |
| Avoid over-drying. | | - |
| | | |
| 14. Remove the tube from the MagNa Stand and add 45 μl of Plasmid Elution Buffer. | 14. Remove | the tube from the MagNa Stand and add $45~\mu l$ of Plasmid Elution Buffer. |
| 15. Carefully resuspend the pellet by gentle pipette mixing. | 15. Carefull | y resuspend the pellet by gentle pipette mixing. |
| | 16. Incubate | the tube at 80°C for 2 - 3 minutes with intermittent tapping. |
| | 17. After in | cubation, place the tube on the MagNa Stand until the solution becomes |
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Note: In the elution step, if magnetic nanoparticles take more than 10 minutes to clear, spin the tube at 14,000 rpm for 5 minutes, place on the magnetic Stand and collect the supernatant containing DNA.



Experimental set up for large culture volumes

| Bacterial culture volume | 5 ml | 10 ml |
|-----------------------------------|----------------|----------------|
| Extraction Buffer Volume (step 2) | 500 μ1 | 1200 μ1 |
| Incubation @ 80°C (step 3) | 5 minutes | 5 minutes |
| MagNa Mix volume (step 6) | 400 μ1 | 800 μ1 |
| Wash Buffer volume (step 9) | 500 μl (twice) | 500 μl (twice) |
| Elution volume (step 12) | 100 μ1 | 150 μ1 |

Troubleshooting Guide

| Observation | Possible causes | Suggested Solution | |
|---------------------|---|---|--|
| Low DNA yield or | Insufficient Cell pellet | Take correct volume of bacterial culture. | |
| Poor Quality | insumercia cen pener | Ensure that OD_{600} is above 1 - 1.5. | |
| | Low copy number plasmids | For low copy number plasmids, bacterial culture | |
| Genomic DNA | volume can be varied up to 2 ml. | | |
| contamination | Prolonged incubation time at 80°C | Ensure that lysis incubation time is followed | |
| | during lysis | accurately. | |
| | Old bacterial cultures Absence of ethanol in Plasmid | Perform experiment with overnight culture of | |
| No plasmid DNA | | bacteria. | |
| No prasimu DNA | | Ensure that correct volume of 98 - 100% Ethanol is | |
| | Wash Buffer | added in the Plasmid Wash Buffer. | |
| | Bacterial culture does not have | Check whether the culture contains plasmid. Ensure | |
| Multiple DNA plasmi | plasmid | that the cells are properly transformed. | |
| _ | Nick formation Plasmid Contamination | Harsh pipette mixing may lead to nick formation. | |
| bands in Agarose | | Pipette mix gently. | |
| gel | | Ensure that the cells are properly transformed with | |
| | riasinu Contamiliation | single plasmid only. | |