# Adjustment of GDS-80 differential needle valve

GDS-80 system is an easy and efficient gene delivery system which can apply on both animal and plant targeting experiments by simply changing the barrel and the pressure setting. The design of GDS-80 is based on the same theory of the Rocket Nuzzle. This innovative design provides enormously advantages of gene transfer assays, not only a simple transfer method, but also a time-saving strategy. Each parameter setting of the GDS-80 is very important, because it may determine the performance of gene delivery. The most important part of parameter setting is the adjustment of the differential needle valve where a delicate spring that controls the gas out put is located inside the main body. All GDS-80 products should be adjusted and calibrated prior to performing the bombardment. Also, after using for a period of time or changing of the pressure setting, the strength of the spring will not be the best condition. It is highly recommended that operators must calibrate GDS-80 system prior to bombardment.

All the setting in this article is the best recommended conditions for operators to perform GDS-80. As operators would like to have different setting of the pressure, different sample volume, different distance of the target, and other factors that would improve the bombardment result, the standard procedures in this article is a reference for adjusting GDS-80 under best working conditions. All the parameters should be settled prior to the calibration, such as the pressure, the proper target spacer, and the optimal loading sample volume.

## MATERIALS

- GDS-80 low pressure gene delivery system
- 99.999% nitrogen or helium gas cylinder firmly chained for safety concerns
- 3 cm and 6 cm target spacers
- 3 mm filter paper
- Coomassie Brilliant Blue (CBB) staining solution (0.1% Coomassie Blue R-250 dissolved in proportion of distilled water/Methanol/acetic acid = 45/45/10)

## PROCEDURES

1. Choose the correct barrel in need and follow the manual to assemble the whole system.
2. Perform the leakage test which is illustrated in the GDS-80 manual.
3. Check the pressure adjustment handle is closed by loosening it counterclockwise first. Also make sure the needle valve on the flow meter is opened by turning it counterclockwise.
4. Open the gas cylinder valve and adjust the pressure adjustment handle to have the desired pressure at the secondary pressure gauge.
5. Fully loosen the differential needle valve and re-tighten it to have recommended setting (as in Table 1) of the valve.

**Table 1.** *Recommended setting of the differential needle valve*

| Pressure setting (psi) | 20 | 30 | 40 | 50 | 60 | 70 |
|---|---|---|---|---|---|---|
| Recommended setting (rounds) | 6.5 | 6 | 5 | 4 | 3 | 2 |
| Setting limitation* (rounds) | < 7 | < 6.5 | < 6 | < 5 | < 4 | < 3 |

*Setting limitation is the maximum setting of the gas output. In order to avoid damaging the GDS-80, do not adjust differential needle valve over the setting limitation and pull the trigger.

6. If the pressure setting is not showed on the recommended setting (Table 1), please find the pressure setting region and tune the differential needle valve gradually of the increasing thrust. For example, if the desired pressure is 36, please tune the differential needle valve from 5 to the maximal 6 rounds.
7. Pull the trigger for few times to check if the pressure keeps fixed.
8. Adjust the needle valve on the flow meter to have stable flow rate at 10~15 L/min for three continuous shots.
9. Apply 10 µl CBB staining solution into the sampling hole. Operators can also refer to Table 2 and find the proper working volume.
10. Fit the GDS-80 muzzle vertically on to the 3 cm or 6 cm target spacers for 4.5 mm

barrel; direct GDS-80 at the 3 mm filter paper for 10.0 mm barrel.

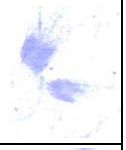
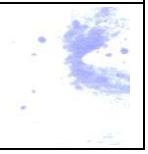
11. Pull the trigger once for 4.5 mm barrel; pull three times for 10.0 mm barrel at different positions on the 3 mm filter paper. Then change the filter paper.
12. Refer to the pattern in Table 3 to confirm your performance. First, check the size of the spots, and then check the particle size of the distribution.
13. If the spreading is not even, make sure it is clean inside the barrel, and then adjust the differential needle valve by fine-tune. (Do not turn the needle valve more than the setting limitation in Table 1.)
14. Repeat step 9 to 13 until the CBB solution is evenly spread.
15. Close the gas cylinder valve first and pull the trigger for few times to exhaust the air between the secondary pressure gauge and GDS-80. And then turn the pressure adjustment handle counterclockwise two rounds to close it.
16. Disassemble the barrel and clean it with 95% EtOH. Allow time to dry it.
17. *Do not tune the differential needle valve once finish the adjustment.* After the muzzle is dry, re-assemble the barrel, and GDS-80 is ready for getting start.

**Table 2.** *Working sample volume range*

| For 4.5 mm barrel | | | | |
|---|---|---|---|---|
| Pressure setting (psi) | 70 | 60 | 50 | 40 |
| Maximum volume (µl) | 16 | 14 | 12 | 12 |
| Minimum volume (µl) | 6 | 6 | 6 | 8 |
| For 10.0 mm barrel | | | | |
| Pressure setting (psi) | 50 | 40 | 30 | 20 |
| Maximum volume (µl) | 12 | 12 | 14 | 14 |
| Minimum volume (µl) | 6 | 6 | 6 | 4 |

## RESULT

**Table 3.** *Reference result of the spots.*

| 4.5 mm Barrel | Best condition (Even) | | Failure (Uneven) |
|---|---|---|---|
| With 3 cm target spacer under 50 psi |  15 mm | |  |
| With 6 cm target spacer under 50 psi |  20 mm | |  |
| 10 mm Barrel (20 psi)* | Best condition (Even) | Workable condition | Failure (Uneven) |
| First shot |  10 mm |  |  |
| Second shot |  |  |  |
| Third shot | |  |  |

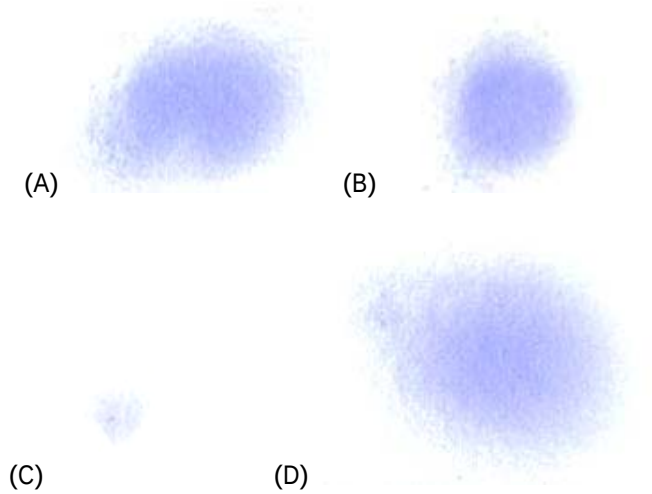



**Figure 1.** *Failure result of the calibration with 50 psi and 6 cm target spacer. (A) Rounded shape with irregular indentation. (B) Rounded shape with uneven particle size. (C) Sample leakage. (D) Not rounded shape.*

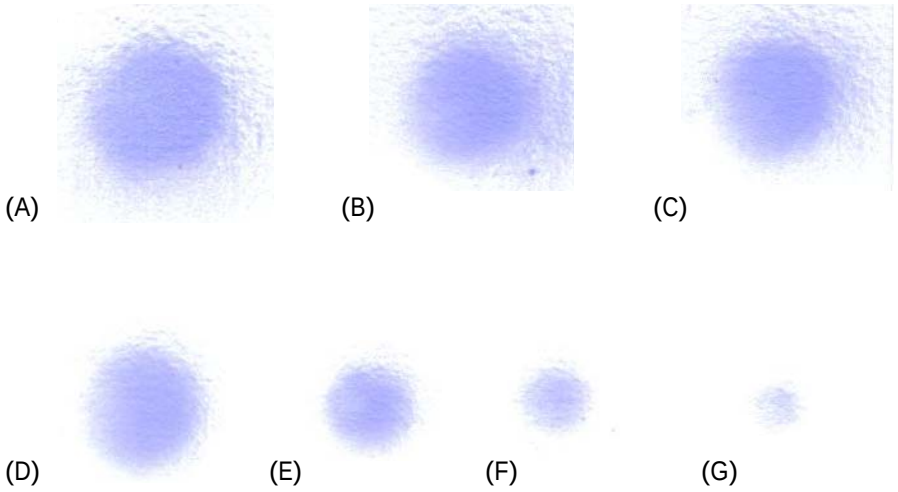



**Figure 2.** *Result of different sample loading volume by using 4.5 mm barrel and 3 cm target spacer. Pressure setting at 50 psi. (A) 20 µl (B) 18 µl (C) 16 µl (D) 10 µl (E) 6 µl (F) 4 µl (G) 2 µl.*

## DISCUSSION

Under the recommended condition of GDS-80 bombardment, the best bombardment result is as in Table 3. Operators should refer to the best condition result to have the perfect tuning of the differential needle valve. In Table 3, the best spot diameter is around 20 mm while using the 6 cm target spacer and 15 mm while using the 3 cm target spacer under the same setting with 4.5 mm barrel at 50 psi and the sample volume is 10 µl. While using of the 10.0 mm barrel, the spot diameter is the same as the barrel. Operators need to check the sample residues within three continuous shots of the 10.0 mm barrel test. If the uneven calibration results happen as in Table 3 and Figure 1, please follow the notification and tuning method procedures as followed:

1. Rounded shape spots with uneven particle distribution (Figure 1 (B)):

   This is caused by over loading of the sample volume, the gas output intensity is lower than required, or sample remaining in the barrel or around the muzzle. The resolutions are:

   a. Please clean the barrel and the muzzle, and then repeat the test again.
   b. Turn the differential needle valve counterclockwise by fine-tune to increase the gas output intensity and repeat the test again.
   c. Make sure to have fixed air flow rate at 10~15 L/min.
   d. Decrease the sample volume for the test.

2. Irregular indentation of the spots (Figure 1 (A)):

   The indentation of the spot is caused by the non-vertical bombardment during the operation or the unclean barrel. Please clean the barrel first, and make sure to put the muzzle vertically toward the target during operation.

3. Sample leakage (Figure 1 (C)):

   Sample leakage will occur when the sample is loaded with bubbles in the sample loading hole, or the sample is not totally loaded into the sample loading hole. Please make sure to have correct loading to prevent sample leakage.

4. Clear or residual spot at the third shot of the 10.0 mm barrel test:

   If the clear or residual spot at the third shot of the test happens, it represents that the gas output intensity is not strong enough. Operators need to increase the gas output intensity by tuning the differential needle valve counterclockwise to solve the problem.

As in Figure 2, the sample loading volume will also affect the spot size and the uniformity of the sample distribution. The best recommended sample volume is using of 10 µl; meanwhile the optimal working sample volume for the bombardment is showed in Table 2. Within these working volume ranges, operators can get the acceptable evenly distribution of the spots.

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