Wealtec Corp.

GDS-80 Low-pressure Gene Delivery System

Installation and Operation Manual
Version 2.4
Item #03150

*This instrument is intended for laboratory use only

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A. Important Notice

Before setting up and operating GDS-80, please carefully read these instructions to get familiarized with the installation and operation process. Instructions should be read by well-trained individuals or by technical people from Wealtec Corp. before operating the instruments. Any improper usage of the instruments may cause damage. Please refer to the safety notice which included in this instrument.

The instruments shall not be modified or altered in any way. Any modification or alteration will void the warranty and the regulatory certifications, as well as create potential safety hazard. Wealtec will not be responsible for any injury or damage caused by using the instruments for any non-intended purpose or injury as a result of modification of the instruments by any person who is not authorized by Wealtec Corp.

The instrument is suitable for life science research use only.

A-1. Warranty

GDS-80 is warranted to be free from defects in materials or workmanship for one year valid from the original invoice date under normal usage. Any defects occurs within warranty period, Wealtec Corp. will repair or replace defective products or parts without extra charge unless the defects arise from conditions which was outlined below. The defects described below are specially excluded from Wealtec warranty policy.

1. Improper operation of the instrument.
2. Repair or modification by any person who is not authorized by Wealtec Corp.
3. Damage caused by any (in)-direct accident, neglect or misuse.
4. Damage caused by disaster.
5. Damage caused by any improper solvents or samples.

A-2. Technical and Service Contact

Most of the operation details are described in this instruction manual to assist and guide operator for an appropriate solution. For any other technical/service questions, please contact your local representative or contact Wealtec international technical/service specialist by E-mail: support@wealtec.com.

A-3. Cautions

Warning!

- Do not put the muzzle direct toward human beings.
- Do not use to deliver any other objects besides nucleic acid and protein.
- Make sure that the gas steel cylinder is stood on the firmly floor and fixed tightly onto the wall, pillar, or laboratory bench.
- For safety concern, please wear goggles, gloves, earplug, and protection coat all the time while operating the GDS-80.
- Make sure to adjust the gas output before every usage.
  (Refer to section D-2)
- Each time after change the gas pressure setting, please adjust the gas output before operation.
- Each time before operating or after finishing the operating of GDS-80, please loosen the pressure adjustment handle on the regulator, to avoid the damage inside the GDS-80.
- For safety concern, please open the gas cylinder valve before operating the GDS-80 and close the valve immediately after finish using it. For any purposes that need to stop operating GDS-80, please also close the gas valve first before all other actions.
- Do not set the pressure over than 75 psi at any time.
- Do not pull the trigger to release the pressure, if the pressure setting is over than 75 psi. Close the gas cylinder valve first. Disconnect quick connector between the hose assembly and GDS-80 and re-connect it again. Repeat above disconnection and re-connection for several times to exhaust the gas pressure until both pressure gauges readouts drop to zero.
- Please note to open the security trigger before pulling the trigger and close it right after pulling the trigger.
- Check if the pressure adjustment handle is loosened by turning counterclockwise every time before open the gas cylinder and after exhaust the gas pressure inside the whole system.
- Do not touch the muzzle at any purpose to prevent any incised wound.

B. Introduction

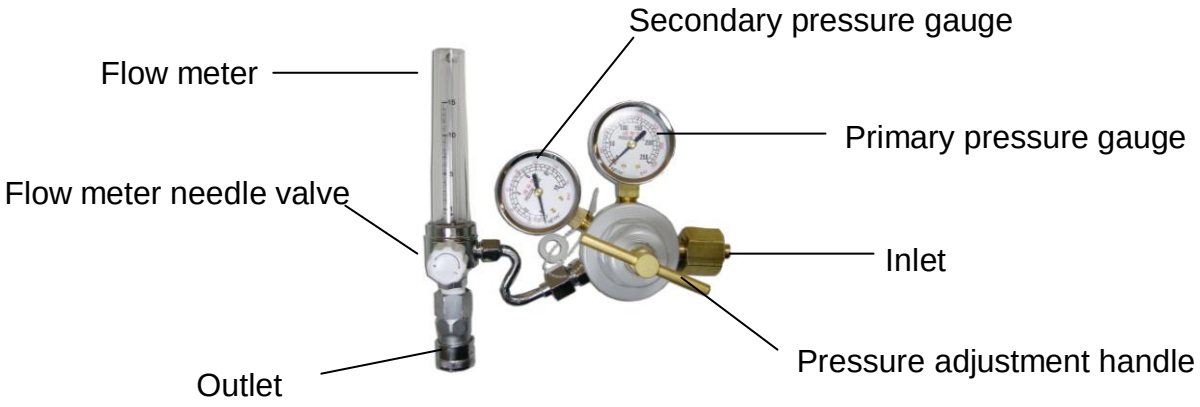
GDS-80 is the gene delivery device using low-pressure helium or nitrogen gases, with or without gold particles to deliver biological materials (e.g. DNA, RNA, and protein) into target cells. Compared to the traditional method, GDS-80 provides a more effective way to save on cost with less sample quantity for both DNA and gold particles.

B-1. Product descriptions

The whole GDS-80 was made in stainless steel, and the system includes pressure regulator (with flow meter), hose assembly, and main body (with the barrel). Helium or Nitrogen gas along with the gas cylinder should be prepared by the customer. The recommended gas purity for both Helium and Nitrogen is 99.999%.

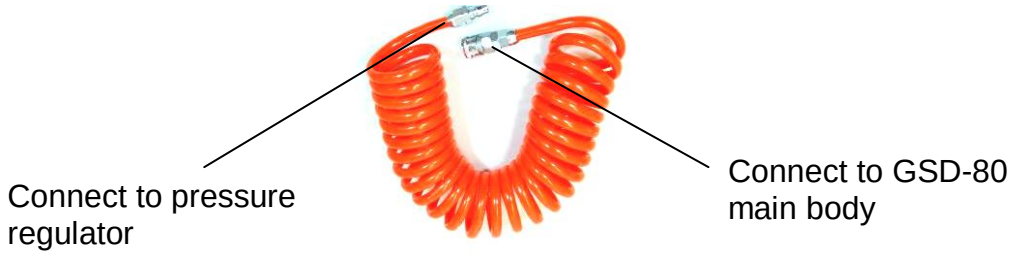
B-1-1. Pressure regulator

There have two pressure gauges, primary and secondary, and pressure adjustment handle on the pressure regulator. The primary pressure gauge is connected to the gas cylinder, which is presenting the pressure from the cylinder. The secondary pressure gauge is connected to the flow meter, and then to the hose assembly on the other side to indicate the gas output pressure. Gas pressure output of the outlet port can be regulated by the pressure adjustment handle, and gas output rate can be regulated by the flow meter needle valve.



B-1-2. Hose assembly

Hose assembly is in charge of the delivery of the gas flow from gas cylinder to the GDS-80. The quick connector design makes connection much easier.

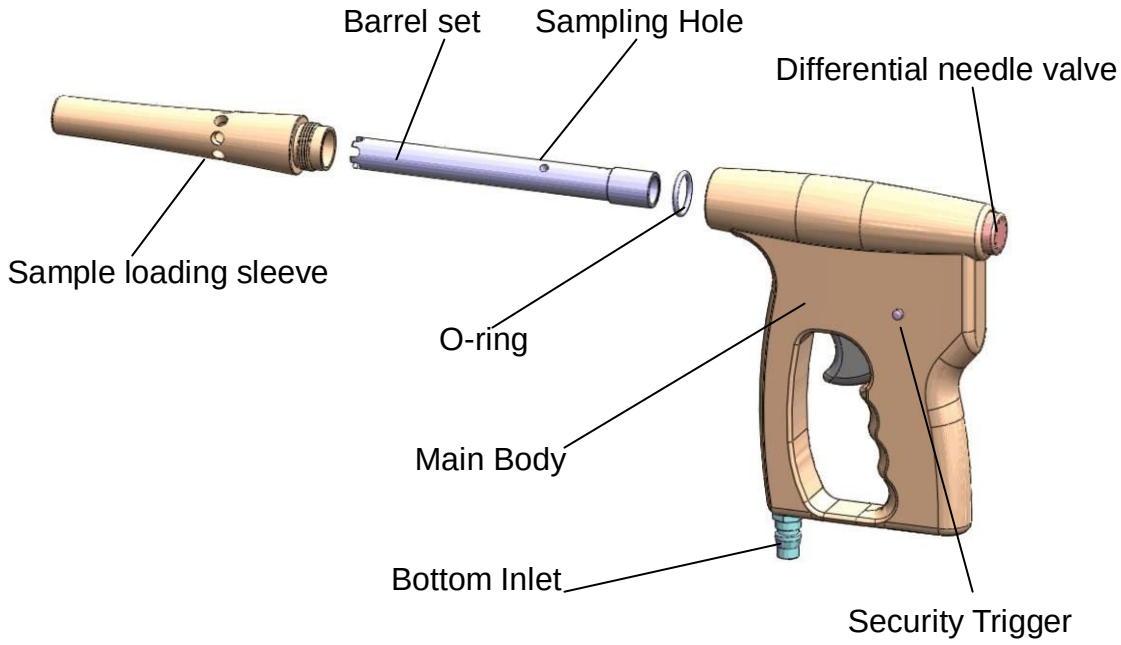


B-1-3. Main body

GDS-80 includes sample loading sleeve, barrel, and main body.

The barrel has two sizes which are applicable to the different applications. The 4.5 mm diameter barrel set is for plant cells and the 10 mm diameter barrel set is specified for animal cells. Sample loading sleeve is a protection cover outside the barrel, and eight sample loading holes on the sleeve for easy sample loading operation. All the equipment here can be sterilized at 121˚C, 1.21 atm, for 15 minutes.

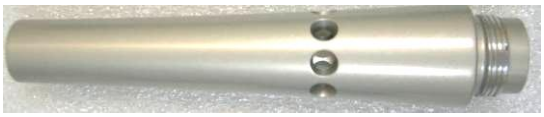
Differential needle valve with engraved scale lines in the back of the main body is used to control the intensity of gas output. The increment of engraved scale lines indicates the higher intensity of gas output. Increase the intensity of gas output by turning valve counterclockwise, and decrease it by turning valve clockwise.

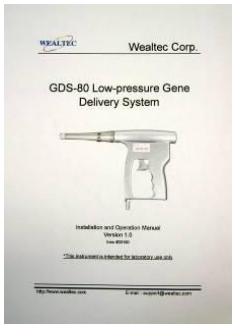


C. Installation of GDS-80

C-1. Package list

Standard package

Item	Quantity
GDS-80 main body 	1
4.5/10 mm diameter barrel set 	1
Sample loading sleeve 	1
Hose assembly 	1 (ID: 8 mm, OD: 12 mm; Full length 4.5 ± 0.2 m)
O-ring 	1
Gas pressure regulator with flow meter 	1 (Outlet: 140 psi Inlet: 3,500 psi) Flow meter: Max. 15 L/min (psi = lb/in^2)

CB-1 Block cooler (1093001) (Optional) 	1
Installation and Operation Manual 	1
Target spacer (optional)  	1 (Height: 3/6 cm, Diameter: 10 cm, used for prevent contamination)
Gold microcarrier (optional) 	1
UTS-10 Universal Target Spacer (optional) 	1 (Include variable distance arm, supporter, pollen cup, shielder, tetraclaw leaf clamp, sample support x2 and sample soft bed x50 for use in live plant transfection)

C-2. Installation

Important Safety Notice：

Please have the nitrogen or helium gas with cylinder properly secured before attaching it to GDS-80 to avoid the possible dangers. Follow the safety instructions provided from the gas supplier for helium or nitrogen installation.

Required Assembly tools and gas

1. Adjustable wrench 2. Box wrench 3. Helium or Nitrogen (99.999%) gas with cylinder

- Unpack the package and take the GDS-80 out of the box.
- Place the GDS-80 on top of the bench, and fix the gas cylinder tightly to the bench to avoid any dangers from collapse risk of the gas cylinder.
- Connect the pressure-regulator with flow meter to the gas cylinder. Make sure the connector of the regulator is tightly connected. The spec of the regulator is illustrated as Fig. C-1. Please connect the regulator to the suitable gas cylinder valve.

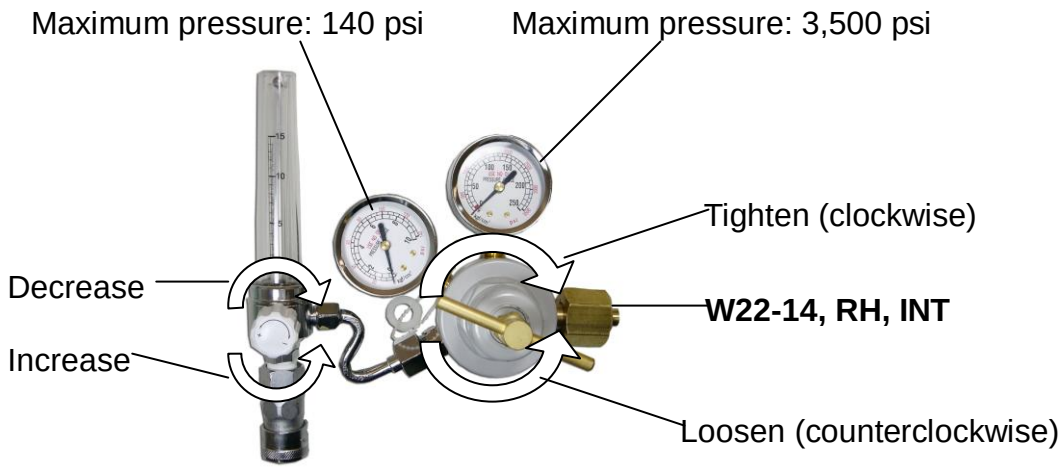


Figure C-1

- The spec of the regulator connector that offered by Wealtec is W22-14, Right Helix, Inner teeth, and it is fitted with JIS-W22 connector on the gas cylinder. If the connector on the gas cylinder is not fit, please find the local standard code of your gas cylinder connector in the below

table, and contact your local distributor to order for the correct connector.

Country	Local Standard Code
Japan	JIS-W22
Taiwan	JIS-W22
China	CGA 540
Hong Kong	BS3
British	BS3
India	BS3
USA	CGA 580, CGA-DISS 718 (>4000 psi)
European Union	CGA 580, CGA-DISS 718 (>4000 psi)
Germany	DIN 6(He), DIN 10(N_2)
Belgium	DIN 6(He), DIN 10(N_2)
Italy	Uni4412(He), Uni4409(N_2)
France	AFNOR Type C

- Connect the hose assembly to the flow meter with quick connector (Fig. C-2, C-3). Pull the hose slightly to check the hose is tightly connected. (Fig. C-4)

Figure C-2

Figure C-3

Figure C-4

- Connect the other end of the hose assembly to the GDS-80 main body with quick connector (Fig. C-5). And also pull the hose slightly to make sure hose is tightly connected.

Figure C-5

- Select the suitable barrel for the experiment. (4.5 mm for plant cells, and 10.0 mm for animal cells) Make sure the O-ring is attached inside the main body without being lost. Assemble the barrel with the sample loading sleeve and tighten it to the GDS-80 main body (Fig. C-6, C-7) by finger-tight. Before screwing it tightly onto the main body, slightly rotate the barrel to adjust the sampling hole to be aimed at the one of loading holes on the sleeve. (Fig. C-8). Do not alter the connection of barrel and sample loading sleeve once it is tightly fixed onto the main body.

Figure C-6

Figure C-7

Figure C-8

D. Operation

GDS-80 is propelled by the low-pressure Helium or Nitrogen gas. The gas flow is compressed inside the main body. While pulling the trigger, the gas flow will be accelerated to an extremely high speed to carry the bio particles to the target cells. Make sure there is no gas leakage within the whole system before operation. After that, follow the calibration procedure as illustrated below to calibrate GDS-80 to the best working condition.

D-1. Leakage test

After the system is completely set up, **Must check** if there's any gas leakage by the following steps:

1. Leakage test between gas cylinder and pressure regulator:
   Step 1: Loosen the pressure adjustment handle first. **(Important step!)**
   Step 2: Open the gas cylinder valve by box wrench to release the helium or nitrogen pressure until the primary pressure gauge reads a pressure value. (More than 1000 psi for plant and 500 psi for animal experiment).
   Step 3: Close the gas cylinder valve (turn the valve clockwise) to ensure the gas will not be released from the cylinder.

Step 4: Read the value on the primary pressure gauge to ensure the pressure value is fixed without leakage (Fig. D-1). If the pressure value doesn't read as a constant and primary pressure gauge is keeping drop to zero while the gas cylinder valve is closed, the leakage may occur. Please check and re-tighten the regulator and repeat the leakage test steps again by the above procedures. Or refer to the trouble shooting listed in section G to solve the leakage problem.

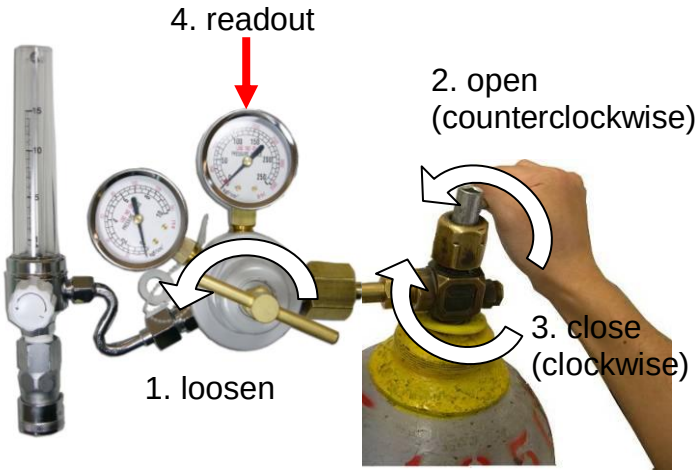


Figure D-1

2. Leakage test between pressure regulator and GDS-80 hose assembly: After the inlet leakage test is done, please continue to proceed with the outlet leakage test by the following steps:
   Step 1: Open the gas cylinder valve again and tighten the pressure adjustment handle to adjust the outlet pressure to 45 ~ 50 psi. (Fig. D-2)
   Step 2: Close the gas cylinder valve to observe the both pressure gauge (Fig. D-3). If the reading pressure is not keep constant but keeping drop to zero, the leakage may occur. Make sure the gas leakage will not cause the pressure drop down more than 100 psi on the primary pressure gauge within 3 minutes. Please be advised to re-connect the hose assembly and the regulator or refer to the trouble shooting listed in section G to solve the leakage problem.

After the leakage test is done successfully, please disconnect the quick connector between the hose assembly and GDS-80 and re-connect it again. Repeat above disconnection and re-connection steps for several times to exhaust the gas pressure until both pressure gauges readouts drop to zero. Then loosen the pressure adjustment handle.

Figure D-2

Figure D-3

D-2. Adjustment of the gas output

NOTE: Make sure to adjust the gas output prior to using the GDS-80 after changing the pressure setting.

After finish the leakage test, open the gas cylinder valve again to check if there has enough gas in the cylinder (more than 1000 psi for plant and 500 psi for animal experiment). Turn the pressure regulator to meet the target pressure according to the experiment requirement. (Fig. D-2, D-3)

Optimize the gas output by following steps:

1. Turn the flow meter needle valve counterclockwise to fully loosen the valve. **Note**: while tuning the flow meter needle valve, adjust the valve slowly at each tuning. (Fig. D-4)

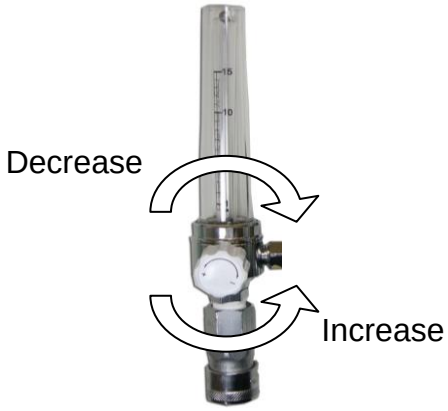


Figure D-4

2. Turn the differential needle valve in the back of GDS-80 main body counterclockwise until it fully loosened. It is normal situation to hear the

gas leakage sound if the pressure setting is over than 40 psi. (Fig. D-5)

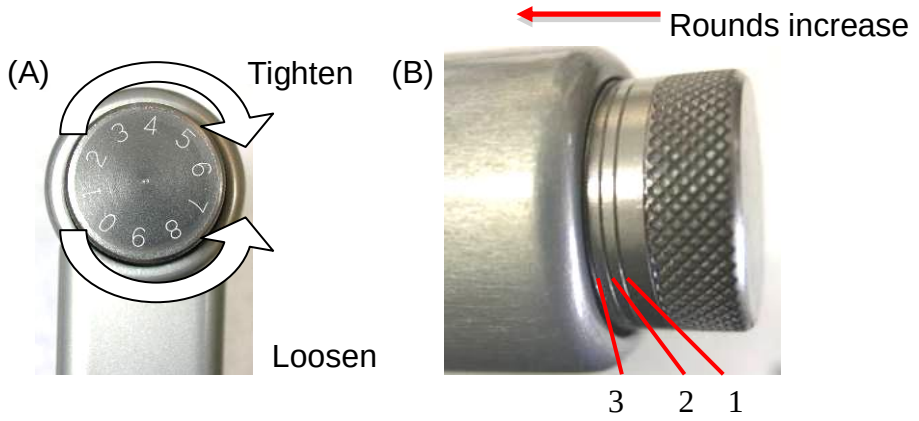


Figure D-5

3. For different pressure settings, the gas output intensity is advised to be adjusted under proper setting by tuning the differential needle valve. The recommended setting is as followed:

Table D-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

*Recommended setting of rounds as illustrated in the Figure D-5 (B). Loosen or tighten the differential needle valve to increase or decrease the setting of rounds.

**Setting limitation is the maximum setting of the gas output for the first time usage. Do not adjust differential needle valve over the setting limitation to pull the trigger to avoid damaging the GDS-80.

4. Refer to Table D-1 to tighten the differential needle valve at recommended setting (as in Fig. D-5). Please check the Table D-1 every time after changing the pressure setting to make sure have proper setting of differential needle valve. When the zero facing upward, it means the start of the rounds.
5. Pull the trigger for three times and make sure the flow rate stays within

the recommended range at 10 ~ 15 L/min by three continuous shots. If it is out of the recommended range, adjust the flow meter needle valve by fine-tune to the desired rate. (Fig. D-4).

6. Check if every shot of gas output gets the same length by listening to the sound. If the length is different, please refer to section G to solve the problem.
7. While adjusting the needle valve on the flow meter, please wait until the floating ball to settle down and then pull the trigger. This adjustment can also help to clean the barrel before operation.
8. **While pulling the trigger, please avoid putting the muzzle direct toward human beings to avoid any possible dangers.**
9. If the gas output intensity is too weak or strong, please refer to section G to solve the problem.
10. After confirm the gas output keeps constant, please continue to calibrate the GDS-80 by the following “Spread even calibration” steps.

D-3. Spread even calibration

After confirm the gas output is fixed, please follow the instruction here to calibrate the spread uniformity to ensure the best working condition of GDS-80. Follow the calibration steps as below:

1. Pull the trigger for few times to check if the pressure keeps fixed after gas output adjustment.
2. The recommended working sample volume is 10 µl. Operator can also refer to the following table to have proper sample volume to apply.

Table D-2. Working sample volume

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

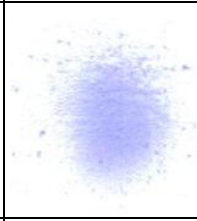
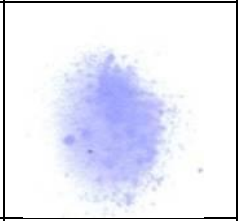
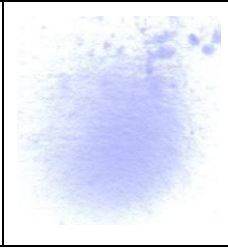
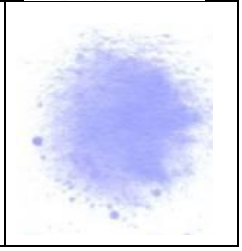
3. Load 10 µl Coomassie Brilliant Blue (CBB) solution into the sampling hole with 10 µl white tip “**vertically**” toward the barrel.

(CBB solution: dissolve 0.05 g CBB in 50 ml 100% EtOH)

4. For the 4.5 mm barrel, attach the GDS-80 muzzle on to the target spacer with 3 mm filter paper matted, and then pull the trigger once toward the filter paper. Then change the filter paper.
5. For the 10.0 mm barrel, put the muzzle direct onto the filter paper, and pull the trigger for three times at different sites on the filter paper. Then change the filter paper.
6. Repeat step 3, 4 or 3, 5 for three times and compare the result with Table D-3.
7. If the result is observed spread unevenly on the filter paper and has no reproducibility in three tests, make sure the barrel is cleaned first, and adjust the differential needle valve by fine tune under the setting limitation. And repeat the calibration steps above again until the CBB solution is evenly spread.

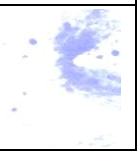
Note: While tuning the differential valve, please make sure to adjust the flow meter needle valve again to ensure the gas flow rate at 10 ~ 15 L/min. Do not adjust differential needle valve over the setting limitation to pull the trigger to avoid damaging the GDS-80.

Table D-3. Reference result of spread even calibration

4.5 mm Barrel	Best condition (Even)	*Workable condition (Little Spots)	Failure condition (Uneven)
With 3 cm target spacer under 50 psi			
With 6 cm target spacer under 50 psi			

*Make sure to load sample with tips vertically to the barrel. The best condition is to have very even spread result in the spot. However, the setting of workable condition is also acceptable in the transfection efficiency.

Table D-3. Cont'd

10 mm Barrel (20 psi)*	Best condition (Even)	Workable condition	Failure (Uneven)
First shot			
Second shot			
Third shot			

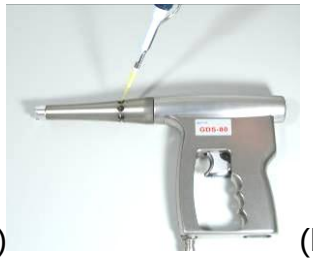
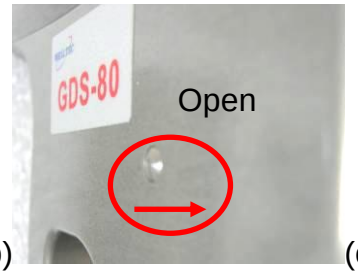
*The result indicates the best working condition and workable condition by using 10 mm barrel under 20 psi.

8. After the calibration, close the gas cylinder valve.
9. Pull the trigger for few times to release the gas within the hose assembly and the GDS-80 main body.
10. Loosen the pressure adjustment handle by turning counterclockwise for at least two rounds.
11. Take apart the sample loading sleeve and barrel and immerse them into the 95% EtOH to clean it.
12. Make sure the barrel is cleaned and dried before use.
13. **Do not adjust the differential needle valve after calibration.**

D-4. Target gene particles delivering

Re-assembling the whole system after the barrel is cleaned and dried. Open the gas cylinder valve and tune the pressure adjustment handle to set the desired pressure as same as used in the spread even calibration step. Pull the trigger for few times to check if the pressure keeps fixed. Put the GDS-80 on the proper site and load the sample into the sampling hole (Fig. D-6(a)). Attach GDS-80's muzzle on the delivering target surface or

the target spacer. Push to open the security trigger before pulling the trigger and push the trigger back from the other side to close right after pulling the trigger (Fig. D-6(b)(c)). Hold the GDS-80 steady and pull the trigger. Wait for the gas flow go through the barrel and pull the trigger again. Repeat pulling the trigger for few times. Notice that different delivery targets have different instructions. Please refer to the section F**.**

(a)  (b) 

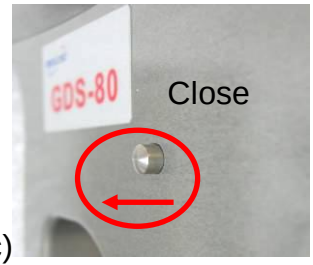
(c) 

Figure D-6

D-5. After use

After delivering of the gene particle, pull the trigger more than two times to have blank shots to clean the barrel avoiding the possible contamination remained in the barrel. Then close the valve of the gas cylinder first (Fig. D-7), and pull the trigger for few times to release the gas within the GDS-80 and the hose assembly. Loosen the pressure adjustment handle (Fig. D-8, Important step!). Disconnect the sample loading sleeve and the barrel to clean them with water. All the equipment here can be sterilized at 121˚C and 1.21 atm for 15 minutes.

Figure D-7

Figure D-8

D-6. Cleaning

D-6-1. Clean between each bombardment

For the same sample bombardment, it only needs to pull the trigger for three times to clean the barrel.

While changing the loading samples (ex, different DNA, RNA, or

protein), please clean the barrel by the following steps to prevent cross contamination of samples.

1. After the last bombardment, pull the trigger for three times.
2. Apply 20 µl sterilized distill water into the sample hole.
3. Pull the trigger for three times.
4. Repeat above procedures for three times.
5. Apply 20 µl 100% EtOH into the sample hole and pull the trigger for three times.
6. Repeat above step for three times.
7. GDS-80 is ready for next sample.

D-6-2. Clean after finish

After finish the use of GDS-80, please clean the barrel by the following steps in order to keep the barrel from cross contamination.

1. Disconnect the GDS-80 system.
2. Wash the barrel and the sample loading sleeve with distill water.
3. Do not wash inside the barrel with tube brush.
4. In case of there have residual gold particles inside the barrel, please immerse the whole barrel in the distilled water in the beaker.
5. Put the beaker into the sonicator.
6. Sonication for 30 minutes.
7. Take out the barrel and wash with distill water.
8. Allow time for drying the barrel for the next experiment.

E. Bombardment Particles Preparation

E-1. DNA-coated microcarrier

Recommended condition for different cell types:

Target sample	DNA amount (per shot)	Sample volume (per shot)	Total DNA amount	Pressure	Distance	Needed Shot(s) (per exp.)
Mouse epidermal cell	1 µg DNA/ 1 mg gold	20 µl	1 µg	30 ~ 40 psi	Attach on the target	1
Plant cell	1 µg DNA/ 0.6 mg gold	10 µl	1 µg	50 psi	3 or 6 cm	1
Maize	0.5 µg DNA/ 0.148 mg gold	10 µl	0.5 µg	50 psi	3 or 6 cm	1

E-1-1. For animal cell —(For five shots)

1. Calculate the needed amount of gold particles and plasmid DNA.
2. Suspend the 5 mg of gold particle in 50 µl sterilized water with vigorously vortexing.
3. Apply 5 µl plasmid DNA solution (1 µg DNA/ µl water) into gold particle solution.
4. Allow Spermidine, $CaCl_2$, 100% EtOH to be placed on ice during process.
5. Add 75 µl of 0.05 M spermidine by drop by drop into the tube and vortex vigorously for well mixed. (Spermidine is mainly used to condense the DNA and apply the positive charge to the gold particle. The volume of spermidine must be more than plasmid DNA solution, and it should be added into a micro-centrifuge tube drop by drop slowly while vortexing. If the DNA is aggregated, please take it for sonication for few seconds until it mixed well.) **Do not premix the spermidine with $CaCl_2$.**
6. Add 75 µl 2.5 M $CaCl_2$ drop by drop into the tube and vortex vigorously to mix it. (The volume of $CaCl_2$ must be more than plasmid DNA solution, and it should be added into a micro-centrifuge tube drop by drop slowly while vortexing. If the DNA is aggregated, please take it for sonication for few seconds until it mixed well.)
7. Place on ice 10 minutes. (It can be put even longer, but do not over than 4 hours).
8. Centrifuge at 6,000 rpm for 1 minutes and discard the supernatant.
9. Apply 200 µl 100% EtOH to suspend the gold particles.
10. Centrifuge at 6,000 rpm for 1 minutes and discard the supernatant.
11. Repeat step 9 ~ 10 for 2 ~ 3 times.
12. Apply 100 µl of 100% EtOH to suspend the gold particles mixture.
13. Aliquot the 20 µl of solution for each shot. Make sure to vortex before each aliquot and pipette the gold-DNA solution few times for well suspension.
14. The DNA-coated gold particle solution is ready for bombardment.
15. Load the adequate gold-DNA solution into the sampling hole.

E-1-2. For plant cell—

Gold particle stock: (60 mg/ml)

1. Weigh out 30 mg gold particles into a 1.5 ml micro-centrifuge tube.

2. Add 1 ml 70% EtOH.
3. Vortex vigorously for 3 ~ 5 minutes.
4. Place at room temperature for 15 minutes.
5. Centrifuge at 6,000 rpm for 30 seconds, discard the supernatant.
6. Add 1 ml sterilized water.
7. Vortex for 1 minute and place the particles at room temperature for 1 minute.
8. Centrifuge at 6,000 rpm for 30 seconds, discard the supernatant.
9. Repeat step 5 ~ 7 three times.
10. Add 500 µl sterilized water for gold particle stock (60 mg/ml).
11. Store gold particle stock at 4°C.

DNA-coated gold particles for 10 bombardments:

1. Take one 100 µl gold particles stock (60 mg/ml) in a 1.5 ml micro-centrifuge tube and add with 10 µl DNA solutions (1 µg/µl).
2. Allow Spermidine, $CaCl_2$, 100% EtOH to be operated under 4^{o}C during process.
3. Add 40 µl 0.1 M spermidine drop by drop into the tube and vortex vigorously for well mixed. **Do not premix the spermidine with $CaCl_2$.**
4. Add 100 µl 2.5 M $CaCl_2$ drop by drop into the tube and vortex vigorously for well mixed Vortex 1 min for mixing well.
5. Centrifuge at 6,000 rpm for 30 seconds and discard the supernatant.
6. Add 200 µl 100% EtOH and vortex for 30 seconds to suspend the gold particles.
7. Centrifuge at 6,000 rpm for 30 seconds and discard the supernatant.
8. Repeat step 6 ~ 7 for 2 ~ 3 times.
9. Add 100 µl 100% EtOH to suspend the gold particles.
10. Aliquot the 10 µl of gene particle solution for each shot. Make sure to vortex before each. Aliquot and pipette the gold-DNA solution few times for suspended well.
11. The gold-DNA solution is ready for bombardment.

E-2. Naked-DNA solution

Recommended condition for different cell types:

Target sample	DNA amount	Sample volume (per shot)	Total DNA amount	Pressure	Distance	Needed Shots (per exp.)
Mouse epidermal cell	7.5 ~ 10 μg DNA/shot	20 μl	7.5 ~ 10 μg	40 ~ 50 psi	Attach on the target	1
Cultured cell line	> 2 μg DNA/shot	10 μl	> 2 μg	20 psi	1 or 2 cm	1

1. Prepare the plasmid DNA to dissolve in (e.g. TE buffer, PBS buffer, sterilized water…etc.) solution into a 1.5 ml micro-centrifuge tube.
2. The DNA solution is ready for bombardment.
3. Load adequate DNA solution for one shot into the sampling hole.

F. Bombardment

F-1. Animal System

F-1-1. Preparation of Target sample

1. Deliver onto mouse epidermal cell:

   Please shave the hair on the mouse's abdomen upon the experiment needs two or three hours prior to bombardment. The operation requires two people, one is for grabbing the mouse, and the other is to operate the GDS-80.

2. Deliver onto cultured cell (Adhesive cell only):

   Subculture the cell into six or twelve well plates that is needed in the experiment for few days before bombardment. Make sure to discard the medium right before the bombardment and refill the fresh medium immediately after that.

F-1-2. Setting up of GDS-80

1. Assemble the GDS-80 system with 10 mm barrel.
2. Setting the delivery pressure according to following recommendations:
   a. Mouse epidermal cell: 30~40 psi for DNA-coated microcarrier
      40~50 psi for Naked-DNA solution
   b. Cultured cell (only for adhesive cell): 20 psi for Naked-DNA solution
3. If the setting of pressure has been changed, the Section D-1 through D-3 must be performed once more before the bombardment. And make sure

the pressure inside the gas cylinder is more than 500 psi.

F-1-3. Performing the bombardment

1. Prepare the gene particles according to Section E.
2. Apply the proper volume into the sampling hole.
3. Attach the muzzle with proper distance from the target cells. The recommended distance from the target cell is as followed:
   a. Mouse epidermal cell: attach on the skin of mouse abdomen
   b. Cultured cell (only for adhesive cell): 1 or 2 cm
4. Pull the trigger for once (refer to Section E-2) to bombard the gene particles.
5. Clean the barrel with three additional shots toward the air.
6. Continue for the next bombardment or finish the operating by proceeding the Section D-5 and D-6.

F-2. Plant System

F-2-1. Preparation

As there have different types of plant samples, the preparations of the target samples are very diverse. Please prepare the samples according to experimental needs. Operators may need to prepare the UTS-10 or 3 cm/6 cm target spacers to help operating the experiment.

F-2-2. Setting up of GDS-80

1. Assemble the GDS-80 system with 4.5 mm barrel.
2. Setting the delivery pressure at 50 psi.
3. If the setting of pressure has been changed, the Section D-1 through D-3 must be performed once more before the bombardment. And make sure the pressure inside the gas cylinder is more than 1000 psi.

F-2-3. Performing the bombardment

1. Prepare the gene particles according to Section E.
2. Apply the proper volume into the sampling hole.
3. Attach the muzzle with the recommended accessories below:
   a. Granule type cells (ex. embryo):

Arrange the cells in the middle of the Petri-dish and covered with 3/6 cm target spacer. It can also use with the UTS-10.

b. Powder type cells (ex. pollen): Refer to the instruction of the UTS-10.

c. Live leaves: Refer to the instruction of the UTS-10.

4. Pull the trigger for one time to deliver the gene particles.
5. Clean the barrel with three additional shots toward the air.
6. Continue for the next bombardment or finish the operating by proceeding the Section D-5 and D-6.

G. Trouble Shooting

1. Gas leakage

   Check the whole connecting line as illustrated in D-1 section with soap water. (Have soap water applied on the connecting line as illustrated in D-1 section to see if the are bubbles generated from the soap water. If yes, it can be assured there's gas leakage.) After revalidation, there have some situations you may encounter as follows:

   - If the connecting line, quick connector, or the pressure regulator have damage and cause the leakage, please contact your local representative or Wealtec international technical/service specialist by E-mail: support@wealtec.com to replace the defect component for further usage.
   - If the connector on the gas cylinder is incorrect, please offer the spec of the connector to your local distributor to get the correct adapter.
   - If the leakage is caused by the un-fitted connecting part, please use the Teflon tape to make it more fitted into the line.

2. The pressure is over than 75 psi on the secondary pressure gauge
   - **Do not release the pressure by pulling the trigger. It will cause the serious damage of the valve inside the main body.**
   - Close the gas cylinder valve first, and disconnect the quick connector on both ends of the hose assembly. Disconnect and connect the quick connector between hose assembly and flow meter for several times to exhaust the gas pressure drop to zero on both pressure gauges.
   - Turn the pressure adjustment handle counterclockwise for two rounds.
   - Reconnect the system, and proceeds the next steps.

3. No air flow or the air flow is too weak, after pulling the trigger.
   - Check the regulator is turned on and the setting pressure is higher than 20 psi.
   - Check if the pressure inside the gas cylinder is enough.
   - Turn the flow meter needle valve counterclockwise to loosen the valve in order to have higher gas output.
   - Turn the differential needle valves counterclockwise by fine-tune to improve the gas output intensity. Notices that do not tune the differential needle valve to have more than setting limitation.
   - After a period time of operation, the springs inside the GDS-80 might get loose. In this situation, it is allowed to tune over the setting limitation only under the condition that the valve inside GDS-80 can close automatically.
   - If there's still no air flow through the system after inspecting above checking points, please contact your local representative or Wealtec international technical/service specialist by E-mail: support@wealtec.com.
4. After pulling the trigger, the air flow can not stop or too strong.
   - Tighten the differential needle valve clockwise to lower down the gas output intensity.
   - It may cause by the over pressure or air flow pushed out. Please lower down the pressure setting.
   - Decrease the flow rate by tuning the flow meter needle valve to shorten the gas output time.
   - If the air flow still cannot stop, please close the gas cylinder valve first and contact your local representative or Wealtec international technical/service specialist by E-mail: support@wealtec.com.
5. While adjusting the gas output, the gas output sound is different between each shot.
   - Tighten the differential needle valve by fine-tune to lower down the gas output intensity.
   - Turn the needle valve on the flow meter clockwise by fine tune, and pull the trigger for several times at the meanwhile to check again. Tune the needle valve until the output gas is stable.
   - If the gas output still not consist after tighten the needle valve, please contact your local representative or Wealtec international technical/service specialist by E-mail: support@wealtec.com
6. The aggregation of the DNA gold particles:

- Please make sure that each vortex step is finished properly.
- Confirm that the DNA sample is diluted within the proper concentration.
- All the aggregation can be solved with brief sonication. Place the micro-centrifuge tube in the sonicator for 5 seconds and then take it out. Repeat for several times to solve the problem.

7. Gold particles cannot go through the skin or organ:
   - Increase operation pressure.
   - Change the barrel to the smaller one.
   - Replace the gold particles with large diameter one.
8. After the delivery on live mouse epidermal cell, it is too wet on the abdomen area:
   - Increase the delivery pressure, ex. from 40 to 50 psi.
   - If it is still wet, please contact your local representative or Wealtec international technical/service specialist by E-mail: support@wealtec.com.
9. Bad result or low expression rate:
   - Confirm that the plasmid DNA can be expressed normally in the target cell.
   - Put the gold particles in the sterilized water to dissolve the DNA on it in order to confirm that the gold particle is made properly with enough DNA on it.
   - Run the PCR experiment to make sure that the plasmid DNA is sent into the cell properly.
   - The expression of the plasmid DNA can also be affected by the quality of target cell.
   - Deliver with over pressure might damage the DNA structure.
   - Please check the pressure in the gas cylinder and make sure it is over than 500 psi for animal experiment and 1000 psi for plant.
   - Turn the differential needle valves counterclockwise by fine-tune to improve the gas output intensity in order to increase the transfection efficiency.
10. Uneven distribution of the gene expression on the target cells:
    - Make sure the GDS-80 is vertically attached on the target cells for bombardment.
    - Check if it is clean inside. If there have some contamination inside, please immerse the barrel into 95% EtOH and put in sonication for 30 minutes to clean it. Do not use the tube brushes to clean inside

the barrel.

- Repeat the Section D-2 and D-3 again.
- If the deliver distribution is still not even after finish all the steps above, please contact your local representative or Wealtec international technical/service specialist by E-mail: support@wealtec.com.

H. Care and Maintenance

1. After every time usage, please fire two to three empty shot to make sure that there has no residues sample inside the barrel.
2. Wash the barrel with water after every time use.
3. Do not wash inside the barrel with tube brush.
4. If there have gold particles coating inside the barrel, immerse it in the 95% EtOH and put in sonication for 30 minutes to clean it.
5. Make sure to sterilized whole GDS-80 before using it to prevent contamination. All of the main body can be sterilized at 121˚C under 1.21 atm for 15 minutes.
6. It is recommended to contact your local supplier for GDS-80 spring maintenance once per year in order to maintain the best working condition of GDS-80.

I. Order Information

Item #	Description
1081001	GDS-80U Low-pressure Gene Delivery Universal System for Plant & Animal, complete with mainbody, 4.5/10 mm diameter barrel set , gas pressure regulator, hose assembly, O-ring, sample loading sleeve, controlled temperature sample preparation device (1093001) and instruction manual. * **Need to order 1081011 Spread even calibration kit for even calibration.**
1081002	GDS-80P Low-pressure Gene Delivery System for Plant, complete with mainbody, 4.5 mm diameter barrel, gas pressure regulator, hose assembly, O-ring, sample loading sleeve, controlled temperature sample preparation device (1093001)and instruction manual. * **Need to order 1081011 Spread even calibration kit for even calibration**.

1081003	GDS-80A Low-pressure Gene Delivery System for Animal, complete with mainbody, 10 mm diameter barrel, gas pressure regulator, hose assembly, O-ring, sample loading sleeve, controlled temperature sample preparation device (1093001) and instruction manual.
1081011	**Spread even calibration kit, complete with 1081201 target spacer x1, 1081202 target spacer x1 and 1035106 Blotting paper, 75x110mm, 50pcs**.

Accessories

1081101	4.5 mm diameter barrel, 1 pc
1081102	10 mm diameter barrel, 1 pc
1081111	GDS-80 system mainbody
1081112	Sample loading sleeve, 1 set
1081113	O-ring, 2 pcs/pk
1081114	Gas pressure regulator
1081115	Hose assembly
1081201	Dish type target spacer for plant, Height:3 cm, Diameter:10 cm
1081202	Dish type target spacer for plant, Height:6 cm, Diameter:10 cm
1081121	UTS-10 Universal Target Spacer complete include variable distance arm, lid stopper, hollow supporter, pollen cup, shielder(20mm x1, 35mm x1, 50mm x1), Tetraclaw leaf clamp, L-type wrench set, sample support x 2 and sample soft bed x 5 for use in live plant transfection.
1081122	LC-10 Leaf clamp complete include variable distance arm, supporter, tetra-claw leaf clamp, sample soft bed x 5 for live plant use only
1081211	Sample Support, Ø=0.28mm, 10ea/pk
1081212	Pollen cup, 1ea
1081213	Tetra-claw leaf clamp
1081214	Sample soft bed x20
1081215	shielder 20mm tall x1
1081216	shielder 35mm tall x1
1081217	shielder 50mm tall x1
1081218	Lid stopper x1
1081219	shielder 20mm tall, pack of 5 x 1081215
1081220	shielder 35mm tall, pack of 5 x 1081216
1081221	shielder 50mm tall, pack of 5 x 1081217
1081222	Pollen cup, pack of 5 x 1081212
1081223	System Stand
1081231	Adapter from W22-14 (Right Helix, Inner teeth) to CGA 540 - China
1081232	Adapter from W22-14 (Right Helix, Inner teeth) to BS3 - Hong Kong, British,

	India
1081233	Adapter from W22-14 (Right Helix, Inner teeth) to CGA 580, CGA-DISS 718 (>4000 psi) – USA, European Union
1081234	Adapter from W22-14 (Right Helix, Inner teeth) to DIN 6(He) -Germany, Belgium
1081235	Adapter from W22-14 (Right Helix, Inner teeth) to DIN 10(N2) -Germany, Belgium
1081236	Adapter from W22-14 (Right Helix, Inner teeth) to Uni4412 (He) - Italy
1081237	Adapter from W22-14 (Right Helix, Inner teeth) to Uni4409(N2)- Italy
1081238	Custom-made Adapter from W22-14 (Right Helix, Inner teeth)-by request

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Wealtec Corp.
1885 Meadowvale Way Sparks, NV 89431
Tel: +1-775-351-2066 Fax: +1-775-351-2077
http://www.wealtec.com E-mail: support@wealtec.com