

Media and Balanced Salt Solutions Preparation from Powder and Concentrates

MAN0006845

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Instructions to prepare Gibco[®] media and balanced salt solutions from concentrates and powder are described below.

General Guidelines

- Use sterile solutions of 1 N NaOH or HCl prepared by membrane filtration using appropriate filters.
- Filter OptiMEM[®] using a low protein binding filter.
- Keep the container with medium covered during stirring and filtration.
- Do not over-mix. Stop stirring when the product is completely dissolved.

Procedure A: Prepare Autoclavable MEM Powdered Medium (1X) (Cat. no. 11700*)

1. To a mixing container that is as close to the final volume as possible, add 950 mL of distilled water.
2. Add powdered medium to room temperature (15°C to 30°C) water with gentle stirring.
3. Rinse inside of package to remove all traces of powder.
4. When completely dissolved, adjust the pH to 4.1 to 4.2 with 1 N HCl.
5. Dispense 95 mL of the liquid into 100-mL bottles. Do not cap tightly.
6. Autoclave the medium for at least 15 minutes at 121°C on a slow exhaust cycle. However, due to equipment variability, we recommend that you qualify your autoclave sterilization cycle.
7. Allow the medium to cool to room temperature.
8. Aseptically add 3 mL of 7.5% NaHCO₃ solution to each bottle. Mix well.
9. Aseptically add L-glutamine and antibiotic solutions, serum, or other supplements, as desired. Mix well.
10. Aseptically adjust the medium to final pH 7.2 to 7.4 by slowly adding, with stirring, 1 N NaOH or 1 N HCl, if necessary.

*For *in vitro* diagnostic use. Caution: Not for human or animal therapeutic use.

Procedure B: Prepare Powdered Leibovitz's L-15 Medium (1X) (Cat. no. 41300*)

1. To a mixing container that is as close to the final volume as possible, add 950 mL of distilled water.
2. Add powdered medium to room temperature (15°C to 30°C) water with gentle stirring. Do not heat the water.
3. Rinse the inside of package to remove all traces of powder.
4. Adjust pH to 7.6 by slowly adding, with stirring, 1 N NaOH or 1 N HCl.
5. Adjust the final volume with distilled water.
6. Process the medium immediately into sterile containers by membrane filtration with a 0.2-µm filter using a positive-pressure system.

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Procedure C: Prepare Powdered Iscove's Modified Dulbecco's Medium (1X) (Cat. nos. 42200*, 12200*)

1. To a mixing container that is as close to the final volume as possible, add 950 mL of distilled water.
2. Add powdered medium to room temperature (15°C to 30°C) water with gentle stirring. Do not heat the water.
3. Rinse the inside of package to remove all traces of powder.
4. Add 3.024 g of sodium bicarbonate/L of medium.
5. Adjust the final volume with distilled water and stir until dissolved.
6. Do not adjust the pH. Keep the container closed until the medium is filtered.
7. Process the medium immediately into sterile containers by membrane filtration with a 0.2-µm filter using a positive-pressure system.

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Procedure D: Prepare Powdered Medium

1. To a mixing container that is as close to the final volume as possible, add 950 mL of distilled water.
2. Add powdered medium to room temperature (15°C to 30°C) water with gentle stirring. Do not heat the water.
3. Rinse the inside of package to remove all traces of powder.
4. Add sodium bicarbonate (see Tables 1, 2 for quantity) to medium.
5. Adjust the pH to 0.2 and 0.3 units below the desired final working pH by slowly adding, with stirring, 1 N NaOH or 1 N HCl.
The pH may rise 0.1 to 0.3 units upon filtration.
6. Adjust the final volume with distilled water.
7. Process the medium immediately into sterile containers by membrane filtration with a 0.2-µm filter using a positive-pressure system.

Table 1 Powdered Media Preparation (Procedure D)

Cat. no.*	NaHCO ₃		Recommended working pH
	g/L	mL/L 7.5% soln.	
11300	0.35	4.7	5.9–6.2
21011	0.85	11.35	7.0–7.4
23600	2.0	26.66	6.8–7.2

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Table 2 Powdered Media Preparation (Procedure D)

Cat. no.*	NaHCO ₃		Recommended working pH
	g/L	mL/L 7.5% soln.	
10012	0.35	4.7	7.0-7.4
11012	0.35	4.7	7.0-7.4
11900	2.2	29.3	7.0-7.4
12000	2.2	29.3	7.0-7.4
12100	3.7	49.3	7.0-7.4
12400	1.2	16.0	7.0-7.4
12500	2.438	32.5	7.0-7.4
12800	3.7	49.3	7.0-7.4
13018	0.85	11.35	7.0-7.4
21700	1.18	15.7	7.5-7.9
22100	2.75	36.6	6.7-7.1
22600	2.4	32.0	7.1-7.4
23400	2.0	26.7	7.0-7.4
31100	2.2	29.3	7.1-7.4
31600	3.7	49.3	7.0-7.4
31800	2.0	26.7	7.0-7.4
32500	2.43	32.5	7.1-7.4
41500	2.2	29.3	7.0-7.4
51800	2.0	26.7	7.0-7.4
61100	2.2	29.3	7.0-7.4

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Cat. no.*	NaHCO ₃		Recommended working pH
	g/L	mL/L 7.5% soln.	
23600	2.0	26.7	6.8-7.2
43000	0.35	4.7	6.1-6.3

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Procedure E: Prepare Powdered Medium (Alternate Recipe)

- To a mixing container that is as close to the final volume as possible, add 950 mL of distilled water.
- Add powdered medium to room temperature (15°C to 30°C) water with gentle stirring. Do not heat the water.
- Rinse the inside of package to remove all traces of powder.
- Add sodium bicarbonate (see Table 3 for quantity) to medium.
- Adjust the pH to 0.2 and 0.3 units below the desired final working pH by slowly adding, with stirring, 1 N NaOH or 1 N HCl.

The pH may rise 0.1 to 0.3 units upon filtration.

- Adjust the final volume with distilled water.
- Process the medium immediately into sterile containers by membrane filtration using a positive-pressure system.

Table 3 Powdered Media Preparation (Procedure E)

Cat. no.*	NaHCO ₃		Recommended working pH
	g/L	mL/L 7.5% soln.	
10012	0.35	4.7	7.0-7.4
11012	0.35	4.7	7.0-7.4
21700	1.176	15.7	7.6-7.9
32500	2.438	15	7.1-7.4
42400	1.125	15	7.1-7.4
52100	3.7	49.3	7.0-7.4

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Cat. no.*	NaHCO ₃		Recommended working pH
	g/L	mL/L 7.5% soln.	
21011	0.85	11.35	7.0-7.4

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Procedure F: Liquid Media: Prepare 1X Solutions from 10X Concentrates

To prepare an acceptable, final 1X solution, perform the following procedure under aseptic conditions. Use Distilled Water (Cat. no. 15230) and 7.5% Sodium Bicarbonate Solution (Cat. no. 25080) for use in this protocol. Also note, because we adjust the pH of all 10X media and 10X balanced salt solution concentrates for solubility, you may need to adjust the pH after the dilution and to add sodium bicarbonate, as appropriate.

- Aseptically dilute 100 mL of 10X concentrate with approximately 850 mL of distilled water.
- Aseptically add the correct amount of 7.5% sodium bicarbonate solution. See Table 4 for concentrations.
- Adjust the pH as necessary with 1 N HCl or 1 N NaOH. See Table 3 for 1X pH values.
- Adjust the final volume with distilled water.
- Dispense the solution into sterile containers. Cap the bottles tightly with sterile closures and store at the recommended temperature (see product label).

Table 4 pH of the Medium after Dilution and Addition of 7.5% Sodium Bicarbonate Solution (Cat. no. 25080)

Cat. no.*	pH of 10X soln.	NaHCO ₃		pH after 1:10 dilution & NaHCO ₃ addition ^a	pH of 1X soln. ^a
		g/L	mL/L 7.5% soln.		
Balanced Salt Solutions					
14060	5.5-6.0	0.35	4.7	7.5-7.8	7.0-7.2
14065	4.4-4.7	0.35	4.7	7.5-7.8	7.0-7.4
14080	4.4-4.7	NA	NA	5.2-5.5	7.0-7.2
14180	5.7-6.0	0.35	4.7	6.0-6.3	7.0-7.2
14185	5.8-6.1	0.35	4.7	6.0-6.3	7.0-7.4
14200	6.7-7.0	NA	NA	7.1-7.4	7.0-7.2
Liquid Media					
11430	4.8-5.3	2.2	29.3	7.6-7.9	7.0-7.4
11825	2.4-2.7	2.2	29.3	7.6-7.9	7.1-7.4
21180	2.0-2.8	2.2	29.3	7.6-7.9	7.1-7.4
21430	4.8-5.3	2.2	29.3	7.6-7.9	7.0-7.4

a: pH range varies depending on the pH of water used in dilution.

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Procedure G: Prepare 1X Dulbecco's Phosphate Buffered Saline from Powder (Cat. no. 21300*, 21600*)

- To a mixing container that is as close to the final volume as possible, add 950 mL of distilled water.
- Add powdered medium to room temperature (15°C to 30°C) water with gentle stirring.
- Rinse the inside of package to remove all traces of powder.
- If calcium chloride is included separately with the medium, add 0.1 g calcium chloride/L of medium.
- Adjust the final volume with distilled water.
- Process the medium immediately into sterile containers by membrane filtration with a 0.2-µm filter using a positive-pressure system.

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Related Products

Product	Catalog no.
Distilled Water	15230
Sodium Bicarbonate	25080
Gibco® Water for Injection (WFI) for Cell Culture	A12873
Gibco® Bottle, 1000 mL	10341-001

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