T Cell Medium (RPMI + 10% FCS + Supplements)

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Purpose

Complete T cell medium based on RPMI 1640, supplemented for optimal survival, activation, and expansion of mouse T cells and NLCs in vitro.

Table of contents

Purpose 1

Linked protocols 1

Working buffer composition 2

Preparation 2

A. Preparation of stock (if applicable) . . . 2

B. Preparation of working buffer . . . 2

Stock solutions required . . . 2

Volume calculations . . . 3

Preparation table . . . 3

Storage and stability 4

Reagent details 4

Safety (brief) 4

Version history 5

Linked protocols

- `NLC-PLATE-STIM`

Working buffer composition

Component	Final concentration	Notes
RPMI 1640	—	Base medium
Fetal calf serum (FCS)	10% (v/v)	Heat-inactivated
Penicillin/Streptomycin	1% (v/v)	100x stock → 1x final
L-glutamine	2 mM	From 200 mM stock; prefer GlutaMAX if medium will be stored at 4°C for weeks — L-glutamine degrades over time
Sodium pyruvate	1 mM	From 100 mM stock; **optional**
beta-mercaptoethanol	50 uM	From 50 mM stock
HEPES	10 mM	From 1 M stock; **optional** but common
MEM Non-essential amino acids (NEAAs)	1% (v/v)	100x stock → 1x final; **optional**

> **Note**
>
> **Sodium pyruvate, HEPES, and NEAAs** are optional but improve viability in longer cultures and under stress conditions. Worth including for multiday assays (>48 h) or when cells will be under metabolic stress (e.g. cytokine stimulation, limiting nutrients).

> **Note**
>
> **Beta-mercaptoethanol** is particularly important for mouse primary cells, which are more sensitive to oxidative stress than human cell lines. Do not omit for primary mouse T cells or NLCs.

> **Tip**
>
> **GlutaMAX vs L-glutamine:** If medium will be stored at 4°C for more than 1–2 weeks, substitute GlutaMAX at the same volume — L-glutamine degrades to pyroglutamate and ammonia over time, which is toxic at high concentrations. GlutaMAX is stable and converted to glutamine by cells.

Preparation

A. Preparation of stock (if applicable)

Not applicable — prepared directly as the working buffer.

B. Preparation of working buffer

Stock solutions required

Reagent	Stock concentration	Dilution to final	Optional
Fetal calf serum (FCS)	—	10% (v/v)	No
Penicillin/Streptomycin	100x	1:100	No
L-glutamine (or GlutaMAX)	200 mM	1:100	No
Sodium pyruvate	100 mM	1:100	Yes
beta-mercaptoethanol	50 mM	1:1000	No
HEPES	1 M	1:100	Yes
MEM NEAAs	100x	1:100	Yes
RPMI 1640	—	Base medium	No

Volume calculations

Dilution logic:

- 10% FCS → 1:10
- 1% Pen/Strep (100x) → 1:100
- 2 mM L-glut from 200 mM → 1:100
- 1 mM sodium pyruvate from 100 mM → 1:100 *(optional)*
- 50 uM beta-mercaptoethanol from 50 mM → 1:1000
- 10 mM HEPES from 1 M → 1:100 *(optional)*
- 1x NEAAs from 100x → 1:100 *(optional)*

Preparation table

Volumes for mandatory components. Add optional supplements (pyruvate, HEPES, NEAAs) by substituting equivalent volume from the RPMI column.

Reagent	10 mL	50 mL	100 mL	200 mL	500 mL
RPMI (mL)	8.69	43.45	86.9	173.8	434.5
FCS (mL)	1.0	5.0	10.0	20.0	50.0
Pen/Strep (uL)	100	500	1,000	2,000	5,000
L-glut / GlutaMAX (uL)	100	500	1,000	2,000	5,000
beta-ME (uL)	10	50	100	200	500
Pyruvate (uL) *opt*	100	500	1,000	2,000	5,000
HEPES (uL) *opt*	100	500	1,000	2,000	5,000
NEAAs (uL) *opt*	100	500	1,000	2,000	5,000

Label with: - *T Cell Complete Medium* - Date of preparation - Expiry (4 weeks after preparation)

> **Tip**
>
> Add beta-mercaptoethanol fresh where possible for optimal T cell viability.

Storage and stability

- Store at **4 °C**, protected from light.
- Use within **4 weeks** after preparation.
- Do not freeze.

Reagent details

Reagent	Supplier	Cat. #	Notes
RPMI 1640	Various	—	Base medium
Fetal calf serum	Various	—	Heat-inactivated
Pen/Strep (100x)	Various	—	Antibiotics
L-glutamine (200 mM)	Various	—	Degrades over weeks at 4°C; substitute GlutaMAX for long-stored medium
GlutaMAX (200 mM)	Various	—	Stable dipeptide alternative to L-glutamine; use at same volume
Sodium pyruvate (100 mM)	Various	—	Optional
beta-mercaptoethanol	Various	—	Handle in hood; essential for mouse primary cells
HEPES (1 M)	Various	—	Optional; buffering capacity for longer assays
MEM NEAAs (100x)	Various	—	Optional; supports viability in multiday cultures

Safety (brief)

- Handle medium aseptically in a biosafety cabinet.

- beta-mercaptoethanol is toxic and volatile; handle in a chemical hood.
- Dispose of expired or contaminated medium as biohazardous waste.

Version history

Version	Date	Author	Changes
v1.0	2026-03-02	Dillon Corvino	Initial T cell medium document
v1.1	2026-04-15	Dillon Corvino	Added HEPES (optional, 10 mM) and MEM NEAAs (optional, 1x) to composition; marked sodium pyruvate as optional; added notes on GlutaMAX vs L-glutamine stability, beta-ME importance for mouse primary cells, and conditions where optional supplements are recommended
v1.2	2026-09-18	Dillon Corvino	Migrated to shared summary:/helper architecture (buffer/protocol redesign).