

B27 Supplement (AKA B22)

Hanna Lab Protocol – Weizmann Institute of Science

Ver. 2 - Last updated: 17/02/2016 (*improved BSA fraction is now recommended, Pipecolic acid preparation is corrected*)

A) B27 supplement contains 21 components, and **100gr BSA Fraction V IgG free Fatty Acid Poor (Invitrogen 30036578 x 1 unit**)** [Total of 22 items, thus we call it B22], assembled in **Neurobasal medium (Invitrogen 21103-049 x 2 units**)**. Order these items from **Invitrogen** to assemble 800ml of B27.

B) For 800 ml of B27 – please order the following **21 items** from **Sigma-Aldrich#**:

1) Catalase - (store at -20 as is)	C40-100MG X <u>1 unit**</u>
2) Glutathione reduced - (store at +4 as is)	G6013-5G X <u>1 unit</u>
3) #Human Insulin - (store as is -20, store aliquots at -80)	91077C-250MG X <u>1 unit</u>
4) Superoxide Dismutase - (store at -20 as is)	S5395-75KU X <u>4 units**</u>
5) Human Holo-Transferin - (store at +4 as is)	T0665-100MG X <u>2 units**</u>
6) T3 - (store as is -20, store aliquots at -80)	T6397-100MG X <u>1 unit</u>
7) L-carnitine - (store at RT as is)	C0283-1G X <u>1 unit</u>
8) Ethanolamine - (store at RT as is)	E9508-100ML X <u>1 unit</u>
9) D+-galactose - (store at RT as is)	G0625-100G X <u>1 unit</u>
10) Putrescine - (store at RT as is)	P5780-5G X <u>1 unit</u>
11) Sodium selenite - (store at RT as is, aliquots at -80)	S9133-1MG X <u>1 unit</u>
12) Corticosterone - (store at RT as is, aliquots at -80)	C2505-500MG X <u>1 unit</u>
13) Linoleic acid - (store as is -20, store aliquots at -80)	L1012-100MG X <u>1 unit</u>
14) Linolenic acid - (store as is -20, store aliquots at -80)	L2376-500MG X <u>1 unit</u>
15) Progesterone - (store at RT, store aliquots at -80)	P8783-1G X <u>1 unit</u>
16) Retinol acetate - (store at RT, store aliquots at -80)	R7882-1G X <u>1 unit</u>
17) DL-alpha tocopherol (vit E) (store @+4 as is, store aliquots at -80)	T3251-5G X <u>1 unit</u>
18) DL-alpha tocopherol acetate (store @RT as is, store aliquots at -80)	T3001-10G X <u>1 unit</u>
19) Oleic acid - (store as is -20, store aliquots at -80)	O1383-1G X <u>1 unit</u>
20) Pipecolic acid - (store as is -20, store aliquots at -80)	P2519-100MG X <u>1 unit</u>
21) Biotin - (store at +4 as is)	B4639-100MG X <u>1 unit**</u>

- Items can be stored as is according to manufacturer instruction (RT, 4C or -20 as indicated), and some should be aliquoted and stored at -80 for future use (**RED**).

- **#Human insulin** from **PROSPEC BIO** can also be used: CYT-270 (250MG X 1 unit)

- ****** Indicates items that will be completely consumed to make a single 800ml batch of B27 supplement, while the rest are sufficient for 2-100 batches ☺.

- This composition is based on attached publically available information (attached PDFs). Hanna group has validated it on human and mouse naïve and primed PSCs

(in comparison to Invitrogen B27). Please note that 19) Oleic acid and 20) Pipelic acid are added by our group since they support pluripotent stem cell growth.

C) Making stocks for future reuse for some of the components:

3) **Human Insulin (Sigma 91077C-250MG or PROSPEC BIO CYT-270 250MG)**

(125mg is needed for 800ml B27)

- Prepare a 25mg/ml stock solution by dissolving 250mg insulin in 10ml 0.005M HCl overnight or even 2 days at 4C. Store in 1ml aliquots in -80C (use 5 vials per 800ml B27).

6) **T3 (Sigma T6397-100MG)** (80µg is needed for 800ml B27)

- Prepare a 2mg/ml stock solution by dissolving 100mg T3 in 1ml DMSO and then in 49ml of Ethanol. Store in 40µl individual aliquots at -80C (use 1 vial per 800ml B27 supplement).

11) **Sodium Selenite (Sigma S5261, 1mg)** (500µg is needed for 800ml B27)

- Prepare a 1mg/ml stock by dissolving the bottle in 1ml dH₂O. Add 500µl per 800ml B27 supplement).

12) **Corticosterone (Sigma P8783, 1g)** (800µg is needed for 800ml B27)

- Prepare a 2mg/ml stock by dissolving 0.1g Corticosterone into 50ml Ethanol. Make 400µl individual aliquots (use 1 vial per 800ml B27). Store at -80C.

13) **Linoleic acid (Sigma L1012-100MG)** (40mg is needed for 800ml B27)

- Prepare a 100mg/ml stock by adding 0.9ml of Ethanol. Make 400µl individual aliquots (use 1 vial per 800ml B27). Store at -80C.

14) **Linolenic acid (Sigma L2376-500MG)** (40mg is needed for 800ml B27)

- Prepare a 100mg/ml stock by adding 4.5ml of Ethanol. Make 400µl individual aliquots (use 1 vial per 800ml B27). Store at -80C.

15) **Progesterone (Sigma P8783, 1g)** (0.252mg is needed per 800ml B27 stock)

- Prepare a 1mg/ml stock by dissolving 10mg Progesterone into 10ml Ethanol. Store at -80C. Make 252µl individual aliquots (use 1 vial per 800ml B27)

16) **Retinol acetate (Sigma R7882-1g)** (4mg is needed per 800ml B27 stock)

- Prepare a 20mg/ml stock dissolving the bottle in 50ml of Ethanol. Make 200µl individual aliquots (use 1 vial per 800ml B27). Store at -80C.

17) **DL-alpha tocopherol (vit E) (Sigma T3251-5G)** (40mg is needed per 800ml B27 stock)

- Prepare a 100mg/ml stock by dissolving the bottle in 45ml Ethanol. Make 400µl individual aliquots (use 1 vial per 800ml B27). Store at -80C.

18) **DL-alpha tocopherol acetate (Sigma T3001-10G)** (40mg is needed per 800ml B27)

- Prepare a 100mg/ml stock by dissolving the bottle in 90ml Ethanol.
Make 400µl individual aliquots (use 1 vial per 800ml B27). Store at -80C.

19) **Oleic acid (Sigma O1383-1G)** (40mg is needed per 800ml B27 stock)

- Prepare a 100mg/ml stock by adding 9ml of Ethanol.
Make 400µl individual aliquots (use 1 vial per 800ml B27). Store at -80C.

20) **Pipecolic acid (Sigma P2519-100MG)** (40mg is needed per 800ml B27 stock)

- Prepare a 50mg/ml stock by adding 2ml of **water**.
Make 800µl individual aliquots (use 1 vial per 800ml B27). Store at -80C.

All stocks are stable up to 2 years at -80C.

Notes regarding B27 variants:

- **Vitamin A free B27 supplement:** remove Retinol acetate (#16).
- **T3 free B27 supplement:** T3 (#6) can be omitted when relevant (e.g. studying neurons from patients/iPSCs with mutations in thyroid hormone processing and signaling).
- **Anti-oxidant (AO) free B27 supplement:** the following five antioxidants: #1, #2, #4, #17, #18 should be omitted.
- **BSA Free B27 supplement:** Eliminate BSA. If needed, Human Recombinant Albumin or Serum albumin can be used instead.

D) Assembling the GOODIES!!!!

Thaw all items (including frozen pre-aliquot stocks) 1 hour before start of work at RT.

For a total of **800ml** of B27 supplement assemble the following 22 ingredients in a **500ml** of Neurobasal medium as a base (Invitrogen 21103-049)

- 1) In an empty and sterile 1L glass bottle insert total of **100gr** of BSA Fraction V IgG free Fatty Acid Poor powder (Invitrogen 30036578) and 500ml of Neurobasal medium. (The remaining 300ml of Neurobasal will be used for dissolving several of the components indicated below)
- 2) Dissolve **Biotin** (1 unit of 100mg purchased from sigma as is) in 10ml of Neurobasal media, and add to mix.
- 3) Dissolve **Catalase** (1 unit of 100mg purchased from sigma as is) in 10ml of Neurobasal media, and add to mix.
- 4) Dissolve all 4 units of **Superoxide Dismutase** in 10ml of Neurobasal medium and add to mix.
- 5) Weigh 40mg of **Glutathione** and directly add to mix.
- 6) Dissolve all 2 units of **Holo-Transferin** in 10ml of Neurobasal medium and add to mix.
- 7) Weigh 80mg of **L-carnitine** and directly add to mix.
- 8) Weigh 600mg of **D-galactose** and directly add to mix.
- 9) Weigh 644mg of **Putrescine** and directly add to mix
- 10) Add 40microL of **Ethanolamine** directly to mix.
- 11) Add 1 vial of **Progesterone** stock (previously made, dissolved in Ethanol and frozen at -80), directly to mix.
- 12) Add 5 vials of **Insulin** stock (previously made, dissolved and frozen at -80) directly to mix.
- 13) Add 1 vial of **T3** (previously made, dissolved in Ethanol and frozen at -80) and directly add to mix.

- 14) Add 1 vial of **Pipecolic Acid** stock (previously made, dissolved in water and frozen at -80), directly to mix.
- 15) Add 1 vial of **Oleic Acid** stock (previously made, dissolved in Ethanol and frozen at -80), directly to mix.
- 16) Add 1 vial of **Linoleic Acid** stock (previously made, dissolved in Ethanol and frozen at -80), directly to mix.
- 17) Add 1 vial of **Linolenic Acid** stock (previously made, dissolved in Ethanol and frozen at -80), directly to mix.
- 18) Add 1 vial of **Retinol acetate** stock (previously made, dissolved in Ethanol and frozen at -80), directly to mix.
- 19) Add 1 vial of **DL-alpha tocopherol (vit E)** stock (previously made, dissolved in Ethanol and frozen at -80), directly to mix.
- 20) Add 1 vial of **DL-alpha tocopherol acetate** stock (previously made, dissolved in Ethanol and frozen at -80), directly to mix.
- 21) Add 1 vial of **Corticosterone** stock (previously made, dissolved in Ethanol and frozen at -80), directly to mix.
- 22) Add 1 vial of **Sodium Selenite** stock (previously made, dissolved in Ethanol and frozen at -80), directly to mix.

FINALE: Add whatever is remaining (leftover) of the 300ml of Neurobasal.

- F1: Gently mix the bottle (no need for pipetting or harsh shaking). 10 times gentle rocking.

- F2: Leave bottle 12h (overnight) at 4C for optimal dissolving (without any shaking, and keep protected from light).

- F3: Next day, make 5ml Aliquots and store at -20 protected from light. (Stable for 1 year at -20). Avoid repeated Freezing and thawing. Mix is too viscous for filtering, but can be filtered upon addition to media later on:

**For Mouse PSCs we use 5ml B27 per 500ml media bottle (1 aliquot).
For Human PSCs we use 10ml B27 per 500ml media bottle (2 aliquots).**