# Ethanol Limitation Carboy: **30**

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*This protocol results in media containing 30 mM Ethanol, 60 mM Carbon*

**Directions for *one* carboy**:

1. Autoclave carboy
2. Measure ~7L Milli-Q water into a "measuring" carboy
3. Add the following to ~900ml in a 1L graduated cylinder and mix well:
    a. 1000x vitamins 10.0 mL
    b. 1000x metals 10.0 mL
    c. Ethanol, 95% 18.3 mL
4. Add contents of cylinder to "measuring" carboy
5. Measure out 1L of 10X MDS into the cylinder and add to the "measuring" carboy
6. Rinse cylinder with Milli-Q water into carboy
7. Adjust volume to 10L
8. Mix for ~5min with magnetic stirrer. Filter sterilize into the autoclaved carboy.

**Directions for making 20L stock 10X MDS, in case you need them:**

- Fill plastic carboy with approx. 17L MilliQ $H_2O$
- Add with constant stirring:
    - $CaCl_2$-$2H_2O$ 20g
    - NaCl 20g

- $MgSO_4$-7 $H_2O$ 100g
- $KH_2PO_4$ (monobasic) 200g
- $(NH_4)_2SO_4$ 1000g

Bring up to 20L with MilliQ $H_2O$

Aliquot into 2L media bottles

Autoclave