

# Stock Solution



TD-S Revision 2.0

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## Proteinase K Stock Solution - 20 mg/ml

### Instructions

1. Measure 5 ml of 200mM Tris Buffer (pH 8.0), RNase free\* (See [Tris Buffer Stock Solution](#) protocol).
2. Add 1.66 mg (3mM) calcium chloride ( $\text{CaCl}_2$ ) to the Tris Buffer and dissolve.
3. Measure 500  $\mu\text{l}$  of the Tris- $\text{CaCl}_2$  Buffer (pH 8.0) into a centrifuge tube.
4. Add 20 mg Proteinase K ([Proteinase K, GoldBio Catalog # P-480](#) [CAS 39450-01-6]).
5. Mix gently to dissolve the Proteinase K.
6. Sterilize the solution with a 0.22  $\mu\text{m}$  filter.
7. Fill to a final volume of 1 ml with 100% glycerol.
8. Mix until completely dissolved.
9. Store in aliquots at  $-20^\circ\text{C}$  for 1 year.

**Note:** Proteinase K interacts with nucleases. To ensure maximum Proteinase K activity, use water that has been treated with 0.1% DEPC ([DEPC, GoldBio Catalog # D-340](#) [CAS 1609-47-8, mw. = 162.14 g/mol]) to make the buffer. Add 1 ml of DEPC to 1 L of  $\text{H}_2\text{O}$ , shake vigorously, and let incubate for 12 hours at  $37^\circ\text{C}$  to inactivate any RNases. Autoclave the solution to inactivate DEPC before making Tris buffer.

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