



Instructions for ^{64}Cu Small BTS Ligand Kits

Reconstitute the ligand vial

Take 1.0 mL of reconstitution solution into the ligand vial aseptically. The addition of reconstitution solution should be quickly as one aliquot. Immediately close the cap and vigorously and vertically vortex mix for **at least 10 seconds**. This results in a 1 mL of isotonic buffered ligand solution. It is extremely important to mix this vial with extreme vigor immediately after addition of the reconstitution solution. Do not shorten the mixing time or use gentle methods such as swirling or shaking

Ordering and Preparation of ^{64}Cu -ATSM

1. Ordering ^{64}Cu sample. Ionic ^{64}Cu sample can be purchased by any preferred supplier, but make sure they provide a specific activity assay for cold copper.
 - a. Specific activity is defined as xxx mCi $^{64}\text{Cu}/\mu\text{g}$ cold Cu, usually 50-200 mCi at EOB (end of bombardment).
 - b. Small H_2PTSM or H_2ETS kit has a capacity of 0.5 μg of cold Cu. And small H_2ATSM has a capacity of 0.1 μg of cold Cu. If the ^{64}Cu specific activity is 100 mCi/ μg at EOB, H_2PTSM or H_2ETS kit can be labeled with less than 50 mCi of ^{64}Cu at EOB but H_2ATSM can only work with <10 mCi of ^{64}Cu at EOB;
 - c. ^{64}Cu is usually prepared in some lower pH solution. 1.0 mL of reconstitution solution contains buffer safe to neutralize up to 125 μL of pH 1 HCl or 12.5 μL of pH 0 HCl. User needs request the pH information for the solution in preparation of ^{64}Cu from the supplier. If possible, request the ^{64}Cu is prepared in <100 μL volume.
 - d. If diluting ^{64}Cu activity should be necessary, normal saline solution is the ONLY solvent that can be used
2. Transfer the desired amount of $^{64}\text{CuCl}_2$ solution into the reconstituted ligand vial then cap the vial, followed by 10 seconds of vigorous mixing. The mixing is very important

to ensure good radiochemical labeling yield. pH for the resultant solution should be 4.7 to 7.8, according to the amount of $^{64}\text{CuCl}_2 \cdot \text{HCl}$ solution used.

QC for Labeling yield

Oasis column preparation procedure:

1. Install Luer/Luer transfer set to bottom of Oasis cartridge.
2. Install a 3 mL syringe (can be reused) to transfer set.
3. Fill Oasis barrel with 1 mL methanol or ethanol and draw through over 10 seconds – **do not take to dryness.**
4. Fill Oasis barrel with 1 mL saline and draw through in similar manner – **do not go to dryness.**
5. Repeat step 4 and then disconnect the 3 ml receiving syringe.
6. Install a freed 3 mL syringe (included) to transfer set and fill Oasis barrel with 500 μL of pure saline containing no preservative agents such as benzyl alcohol.

Radiochemical purity assay can be preformed by either of the following methods:

Note: Make sure that any saline used in the Q/A does not contain Benzyl alcohol as preservative.

Quick radiochemical purity assay:

1. Dispense small volume (such as $<10 \mu\text{L}$) of the labeled sample into barrel of prepped Oasis cartridge. Add 500 μL of saline.
2. Draw labeled sample through resin bed into 3 mL syringe using continuous flow over 10 seconds load time. **Do not take to dryness.**
3. Dispense 1 mL saline into barrel and draw into the same syringe at similar rate.
4. Remove 3-mL syringe, cap and place in a plastic bag.
5. Place the Oasis cartridge in a separate plastic bag.
6. Assay the two bagged items for relative ^{64}Cu activity.
7. If necessary measure background (BG).
8. Subtract BG count from the two sample counts
9. Compute the untrapped fraction and the trapped fraction as follows. C_1 = cartridge count and C_2 = Syringe. Untrapped (ionic) fraction = $C_2/(C_1+C_2)$. Trapped Fraction = $C_1/(C_1+C_2)$.

Complete radiochemical purity assay:

1. Dispense small volume (such as <math><10\ \mu\text{L}</math>) of the labeled sample into barrel of prepped Oasis cartridge. Add 500 μL of saline.
2. Draw labeled sample through resin bed into 3 mL syringe using continuous flow over 10 seconds load time. **Do not take to dryness.**
3. Dispense 1 mL saline into barrel and draw into the same syringe at similar rate.
4. Repeat step 3.
5. Remove 3-mL syringe, cap and place in a plastic bag (C2).
6. Install a freed 3 mL syringe to transfer set.
7. Add 1 mL of ethanol into barrel and draw into the 3 mL syringe over 10 seconds load time. **Do not take to dryness.**
8. Repeat step 7 for two more times.
9. Remove 3-mL syringe, cap and place in a plastic bag (C3).
10. Place the Oasis cartridge in a separate plastic bag (C1).
11. Assay the three bagged items for relative ^{64}Cu activity.
12. If necessary measure background (BG).
13. Subtract BG count from the three sample counts
14. Compute the ionic fraction and the labeled fraction as follows. C1 = cartridge count and C2 = aqueous saline syringe and C3 = ethanol syringe. Untrapped (ionic) fraction = $C2/(C1+C2+C3)$; Trapped Fraction = $(C1+C3)/(C1+C2+C3)$; Recovered Fraction = $C3/(C1+C2+C3)$.