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SepSphere™ Peptide Immobilization Kit via Maleimide (CM71301) User Reference Guide

Contents

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Important Notes & Contact Information

READ BEFORE USING ANY RESOURCES PROVIDED HEREIN

The information provided in this document and the methods included in this package are for information purposes only. CellMosaic provides no warranty of performance or suitability for the purpose described herein. The performance of this kit in labeling may be affected by many different variables, including but not limited to the purity and complexity of the starting materials, differences in preparation techniques, operator ability, and environmental conditions.

Sample data are provided for illustration and example purposes only and represent a small dataset used to verify kit performance in the CellMosaic laboratory. Information about the chemicals and reagents used in the kit are provided as necessary.

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Kit Components

This kit provides materials to immobilize any thiol-containing peptide onto maleimide-activated agarose beads.

⚠ Upon receipt, please remove **Box 1** and store in a refrigerator at 2-8°C.
Store **Box 2** at room temperature.

	Name	Part #	Quantity	Storage condition
Box 1	Maleimide Agarose Beads in a 5 mL centrifuge tube	CM71604	5 mL settled down beads after swelling	2-8°C
	Buffer A (**indigo label**)	CM02005	55 mL	
	Solution A (**purple label**)	CM01007	2.5 mL	
	Storage Buffer (0.02% NaN_3 in PBS buffer) (**grey label**)	CM02008	55 mL	
Box 2	Column Set	CM03SC7	1	RT
	Centrifuge Tube 15 mL	N/A	1	
	Centrifuge Tube 50 mL	N/A	1	
	Stirrer	CM03PT1	3	
	Airtight Syringe 20 mL	CM03SR1	1	
User Material	Cys-Peptide	NOT PROVIDED (User Supplied Material, **1 to 20 mg**)		

Cys-peptide Amount: This kit uses a maleimide agarose with 5–15 µmole of maleimide groups per mL of settled down beads. 1–20 mg peptide will correspond to 0.4–8 µmole peptide per mL of settled down beads if the MW of the peptide is 2500 Da. You can add more than 20 mg of peptide to target higher loading if you have enough peptide.

Peptide Loading Optimization: The maleimide thiol coupling reaction is very efficient. Peptide loading will be determined primarily by the amount of peptide added during the immobilization if the amount of peptide added is less than the amount of maleimide loaded. You can experimentally measure the peptide loading by checking the % of peptide consumed during immobilization (See HPLC or UV sampling and calculation in **Step B2** and **B5**).

Safety Information

Warning: some of the chemicals used can be potentially hazardous and can cause injury or illness. Please read and understand the Safety Data Sheets (SDS) available at CellMosaic.com before you store, handle, or use any of the materials.

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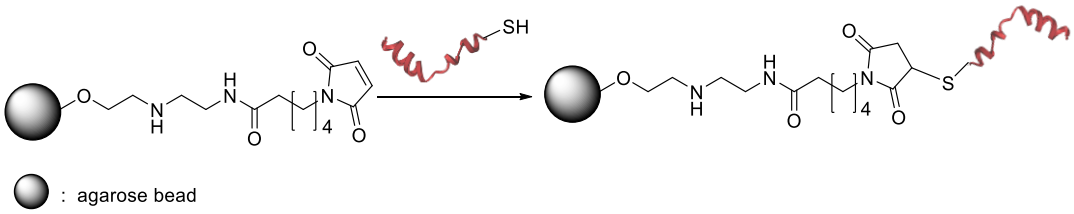
Labeling Chemistry

CellMosaic designed this kit to work with thiol-containing peptide. If your peptide does not have a free thiol for immobilization, you can order a peptide with a Cys placed at either the *C*-terminus or *N*-terminus. The kit provides SepSphere™ agarose beads derivatized with maleimide functional groups (5–20 µmol maleimide per mL of resin). Using the kit components, the customer immobilizes the thiol-containing peptide onto agarose beads in one step following CellMosaic's standard protocol.



Scheme 1: Immobilization of peptide onto agarose via maleimide groups

Key features of this SepSphere™ immobilization kit:

- Minimum requirement for immobilization set-up
- All reagents, buffers, and plasticware are included
- Modestly activated maleimide agarose
- One step immobilization with very mild reaction conditions
- Easy preparation with air push mechanism for washing: less than 1 h hands-on time
- Stable linkage (no leakage of the peptides from the resin under acidic or basic conditions)
- Options to choose tailored custom services at CellMosaic prior to and after conjugation in your lab:
    - Prior to conjugation, you can supply your peptide information when you place your order and CellMosaic will give recommendations for the conjugation if your peptide has special features (complementary service);
    - After conjugation you can choose to send your sample to CellMosaic for HPLC analysis of the peptide samples for determination of the peptide loading.

Specification of the Solid Support

- Functional groups: maleimide
- Matrix: 4% beaded agarose (source: GE Life Sciences, Sepharose® 4B)
- Particle size: 45-165 µm
- Mean bead size: 90 µm
- Ligand concentration: 5–15 µmol maleimide per mL agarose

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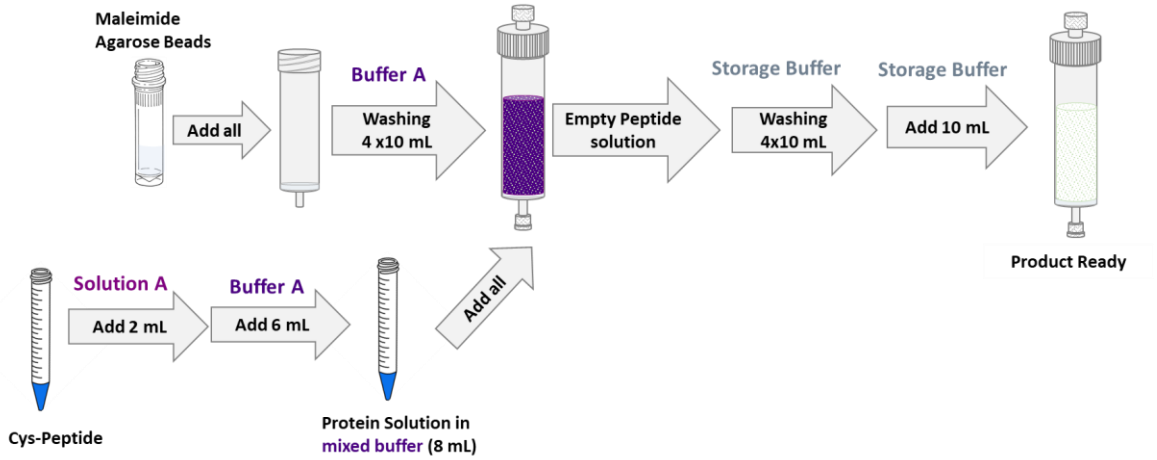
Specification of the Immobilized Product
• Immobilized chemistry: immobilized via thiol ether bond • Chemical stability: stable • pH stability: stable
Requirement for Peptide:
• Cys-containing peptide. If your peptide does not have a Cys, add a Cys at either the *C*-terminus or *N*-terminus • HPLC purified and lyophilized, stored at ≤-20°C as lyophilized solid • HPLC purity: >90% pure by C18 HPLC • Total amount: 1–20 mg • Does not contain any other reducing reagent, such as DTT or mercaptoethanol

Protocol



Scheme 2. Schematic diagram of the workflow for peptide immobilization starting with 5 mL of maleimide agarose beads.

1. Lab Instrumentation Needed

- Vortex mixer
- Centrifuge for 15 mL and 50 mL (preferably refrigerated)
- IEC clinical centrifuge for quick spin (if possible)
- Pipettes and tips
- Timer
- Nutating mixer or 3-D rocker
- Support, clamp, and beaker for waste collection

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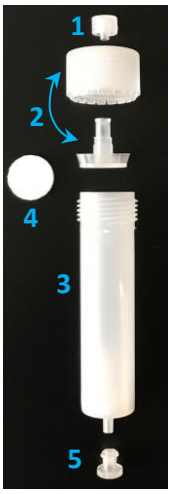
2. Immobilization Lab Techniques at CellMosaic (for reference)

CellMosaic has a universal workflow and kit design that allow immobilization of small molecules and large biomolecules, washing of unreacted starting materials, and column packing all in one column with simple set-up in any lab. The immobilization efficiency in the column is the same as if performed in a regular reaction vessel. The washing is performed with a simple manual air push mechanism using a syringe and requires no gas/air flow or vacuum. The following table outlines the kit component design and key techniques used at CellMosaic for immobilization. Please refer to these key techniques while performing the immobilization according to the user manual.



Column design and setup:

Design: Column comprises five pieces (see left picture)

1. **Male Luer lock cap** (referred to as **top cap** in the protocol and is used during the mixing/nutation).
2. **Column top with Luer attachment** (referred to as **column top** in the protocol). The Luer attachment is for a tight seal and for attachment of the syringe for washing. Column top is removed for addition of buffer and stirring.
3. **Column body containing one polypropylene frit at the bottom** (referred to as **column** in the protocol).
4. **One extra frit for column packing after immobilization** (referred to as **frit** in the protocol).
5. **Bottom female Luer lock plug** (referred to as **bottom plug** and is used during mixing/nutation and storage).

Setup (see right picture): Securely attach the column to a support stand, lab frame, or any support rod and place a beaker or a flask under the column for waste collection.

Use of buffer(s) with resin in column

Design: All of CellMosaic's buffers are supplied in small tubes and are easy to pour directly into the column. Washing buffers generally come with the exact amount needed for optimal washes. The combined wash-volume is fixed, regardless of how much you pour each time.

Procedure:

1. Remove the top cap.
2. Unscrew the column top and bottom plug.
3. Place them on a clean surface for reuse later.
4. Pour 8-10 mL of **any buffer** into the column containing resin for immobilization (~2 volumes of the resin bed).

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Mixing resin in column

Design: The kit comes with one or a few blue polypropylene stirrers of the right length for individual columns. The stirrer is resistant to general solvents, and resin in general will not stick to the stirrer.

Procedure for mixing resin with washing buffer: Mix the resin with buffer using a clean stirrer for ~5-10 seconds. Make sure the stirrer goes into the bottom of the column and the resin is mixed well. Then remove the stirrer without any resin adhering to it. Set aside the stirrer for repeated washing.

Procedure for mixing resin with reaction buffer: Mix the resin with buffer using a clean stirrer for 10-30 seconds. Make sure the stirrer goes into the bottom of the column and the resin is mixed well. Then remove the stirrer without any resin adhering to it. Dispose of the stirrer as solid waste. **Note:** For some reactions, gas/air bubbles may be generated. Stir slowly and continuously until most of the air bubbles are gone before capping it for mixing (nutation).

Washing resin after stirring

Design: The kit comes with one 20 mL airtight syringe with slip tip that can be used to push air through the column.

Procedure for washing:

1. Attach the column top to the Luer attachment after stirring.
2. Draw 20 mL of air into the syringe and attach the syringe to the column top.
3. Push the air through the column.
4. Once the plunger reaches the bottom of the syringe, detach the syringe. Repeat the air drawing and purging process. In general, there will be approximately 1.2 mL of residual liquid after several pushes. It is OK to go on to the next washing step.
5. Remove the column top, fill with buffer, and repeat the washing and purging process.

Procedure to remove residual liquid: If residual liquid is to be removed for certain reactions (see individual protocol), use this additional step after air purging with the syringe. Place the column into a 50 mL centrifuge tube with the column top attached without the top cap. Create a counterbalance with a 50 mL centrifuge tube with water in it. Place both tubes into a quick spin centrifuge, such as an IECclinical centrifuge. Set the spin to the maximum setting and spin for 15-20 seconds. Alternatively, you can use conventional centrifuge equipment and spin at ≤750 x g for 1 minute.

Column setup for immobilization:

After adding reagents per the protocol, attach the column top and securely cap/plug the column top and bottom. Check to make sure everything is tight and there is no leak before putting the column in a nutator. If you do not have a mixer or nutator, leave the column open and stirrer in place. Stir the resin every 5-10 minutes for the specified time.

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Storage buffer and others:

The kit comes with standard storage buffer (1x PBS with 0.02% NaN_3). If you need to use your own buffer, substitute your buffer during the last washing.

Column packing:

Design: You can pack the resin in the column once the immobilization is done. A separate polypropylene frit is included in the kit for column packing. Otherwise, you can store the resin in the column and pipette out as needed for usage.

Procedure:

1. Remove the top cap. Unscrew the column top and bottom plug.
2. Fill the column with deionized water or buffer up to the top and wait a few minutes so that most of the resin settles down.
3. Wet the frit with deionized water before placing the frit inside.
4. Remove the black rubber cap in the plunger of the 20 mL syringe.
5. Use the plunger to push the frit inside the column and all the way down to the top of the resin. Make sure the pressure is even and the frit is flat and not tilted. There should be no air in between the frit and the resin before pushing the frit all the way down to the top of the resin.

6. Once the frit reaches the top of the resin, use slight pressure to make the frit tight.
7. Attach the column top with Luer attachment securely and cap the top.

The column is ready to use. You can use gravity flow or attach the column to a peristatic pump with Luer lock adapters.

Protocol for immobilization starts on next page

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3. Preparation of Agarose Beads for Immobilization

Items needed: Maleimide Agarose Beads (CM71604), Column Set (CM03SC7), Airtight Syringe 20 mL (CM03SR1), Stirrer (CM03PT1), Buffer A (CM02005, indigo label), 50 mL centrifuge tube.

A1. Securely attach the **Column** to a support stand, lab frame, or any support rod and place a beaker or a flask under the column for collection. Remove the top cap. Unscrew the column top and bottom plug. Place them on a clean surface for reuse later.

A2. Slightly centrifuge the tube containing agarose beads to ensure no bead is in the cap before opening. Pour all the beads into the **Column**.

A3. Locate **Buffer A** (indigo label) and pour 8-10 mL of **Buffer A** into the column. Stir the resin with a clean stirrer for 5 seconds and let it stand for 5−10 minutes to swell. Set aside the stirrer for later use.

A4. Attach the column top. Use the 20 mL syringe to push the liquid out.

A5. Remove the column top and repeat the washing (**Steps A3** and **A4**) three times. Dispose of the stirrer.

A6. (Skip this step if you are not planning to measure the peptide loading) Place the column into a 50 mL centrifuge tube and use a quick spin centrifuge, such as an IEC clinical centrifuge. Counterbalance with a 50 mL centrifuge tube containing water. Set the spin to the maximum setting and spin for 15 seconds (After emptying the residual liquid, the 50 mL centrifuge tube will be set aside and used in **Step B6** for recovering the unreacted peptide).

4. Preparation of Peptide Samples and Immobilization

Items needed: Buffer A (CM02005, indigo label), Solution A (CM01007, purple label), 15 mL centrifuge tube

> ⚠ The following steps (**B1–B3**) are to be performed without any break. Cys-peptide, once dissolved in solution, tends to oxidize quickly and should be used immediately. Do not store any Cys-peptide solution for later usage.

Total amount of peptide used for the immobilization is 1–20 mg to target 0.2–4 mg peptide per ml of settled resin. For loading >4 mg/ml, you can weigh more peptide.

B1. Weigh 1−20 mg of **Cys-peptide** quickly into a 15 mL centrifuge tube.

Note: the peptide in general is static charged. Use the tip of a glass Pasteur pipet to weigh the peptide if possible. It may be difficult to obtain the exact weight. Any amount between 1 and 20 mg or more is acceptable (no need to adjust the volume of the solvent).

B2. Add 2 mL of **Solution A** (purple label) to the centrifuge tube containing Cys-peptide from **Step B1.** Vortex for 30 seconds and centrifuge the tube.

Open the cap and add 6 mL of **Buffer A** (indigo label) to the tube. Vortex for 30 seconds or sonicate for a few minutes to ensure all the solid is dissolved.

Discard any unused **Solution A** as hazardous chemical waste **until the experiments are done**.

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Tip for solubility check (Step B2): It may take a while for your peptide to fully dissolve. In general, most of the peptide should be able to dissolve in this mixed solution system. Check the bottom of the centrifuge tube to ensure the solution is clear and free of any solid residue. If some solid remains after a few minutes, centrifuge the tube, and pipette the supernatant for the next step.

Tip for opening centrifuge tube after vortexing: Always spin the tubes to ensure no liquid is in the cap.

HPLC or UV Sample (B2) for Peptide Loading Measurement (Optional): If your peptide contains any of the following amino acid residues (Phe, His, Trp, or Tyr), you can use UV to determine the loading. Otherwise, please use the HPLC analysis.

- Transfer 50-100 µL of the peptide solution from **Step B2** to a 1.5 mL centrifuge tube and label it as **sample B2** (The volume taken depends on the peptide concentration and UV cell volume. The dilution factor will depend on the extinction coefficient of the peptide and the peptide concentration).
- For UV measurement with 1 cm path length, dilute the sample 2–30 times in **Buffer A** (final concentration is ~50 µM for a 3000 Da peptide). Record the dilution factor DF(**B2**) and UV absorbance of the peptide at 254 nm (A(**B2**)). Remember to use **Buffer A** as a blank for subtraction.
- For HPLC analysis, dilute the sample to ≤1 mg/mL in **Buffer A** (detect at 254. If your peptide does not have Phe, His, Trp, or Tyr residues, you can detect the peptide at 220 or 205 nm). Record the dilution factor DF(**B2**) and HPLC area of the peptide peak (A(**B2**)).

B3. Securely plug the end of the column from **Step A5** or **A6** with the bottom plug and immediately add the peptide solution from **Step B2.**

B4. Stir the resin solution for 10-30 seconds with a new stirrer until there are few air bubbles. Securely cap the column with the top cap. Dispose of the stirrer.

B5. Nutate the column at RT for 2–4 h (overnight is fine).

In-process Sample Analysis by UV/Vis and HPLC (Optional):

- Pipette 500 µL of liquid from **Step B5** into a 1.5 mL centrifuge tube (let the resin settle before removing the liquid from the top, avoid taking the resin).
- Centrifuge at 14,000 x g for 1 minute.
- Remove 50–100 µL of the supernatant carefully from the top and dilute in **Buffer A** the same factor as DF(**B2**). Put the rest of the sample back to the reaction mixture. Record the UV absorbance of peptide at 254 nm or HPLC peak area of the peptide A(**B5**).

Percentage of the peptide consumed:

$$\% = \left[1 - \frac{A(B5)}{A(B2)}\right]$$

A(**B5**): UV absorbance or HPLC peak area of the peptide from **Step B5**
A(**B2**): UV absorbance or HPLC peak area of the peptide from **Step B2**
Both samples are measured under the same condition using the same dilution factor and UV cell.

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Reaction time: In general, after 2 h at RT, 80% of the peptide will be consumed depending on your initial peptide concentration. Some Cys-peptides may be dimerized or oxidized after prolonged reaction times. You can continue letting the resin nutate at RT and see if there are any changes to the peptide concentration. Remember to take a new sample for UV reading before moving on to the next step for calculating the loading.

Calculation for Loading:

$$Peptide\ loading\ (mg\ per\ mL\ resin) = Peptide\ amt\ (mg) \times 0.2 \times \left[1 - \frac{A(B5)}{A(B2)}\right]$$

$$Peptide\ loading\ (\mu mole\ per\ mL\ resin) = \frac{Peptide\ amt\ (mg)}{Peptide\ MW} \times 200 \times \left[1 - \frac{A(B5)}{A(B2)}\right]$$

B6. Attach the column back to the lab stand. Place a 50 mL centrifuge tube under the column to collect unreacted peptide solution. Open the bottom plug. Push the liquid out of the column using the syringe. Wash the end plug with deionized water for reuse.

5. Washing and Storage

Items needed: Stirrer (CM03PT1) and Storage Buffer (CM02008, grey label).

C1. Remove the column top and pour in 8-10 mL of **Storage Buffer.** Stir the resin solution for 10-30 seconds with a new stirrer, then screw the column top back on and push the liquid out of the column using the syringe.
C2. Repeat washing (**Steps C1**) 3 times. Dispose of the stirrer.
C3. Securely plug the end of the column and add 5-10 mL of Storage Buffer. Screw the column top back on and securely cap the column. Store at 2-8°C in a refrigerator. Do not freeze.

The Agarose Beads are Ready for Your Experiment

Specification for your product

Matrix: 4% beaded agarose (source: GE Life Sciences, Sepharose® 4B)
Particle size: 45 – 165 µm
Ligand concentration: calculated in **Step B5**. If you do not perform the UV measurements, assume 70-80% of peptide consumed and use that value for the calculation.

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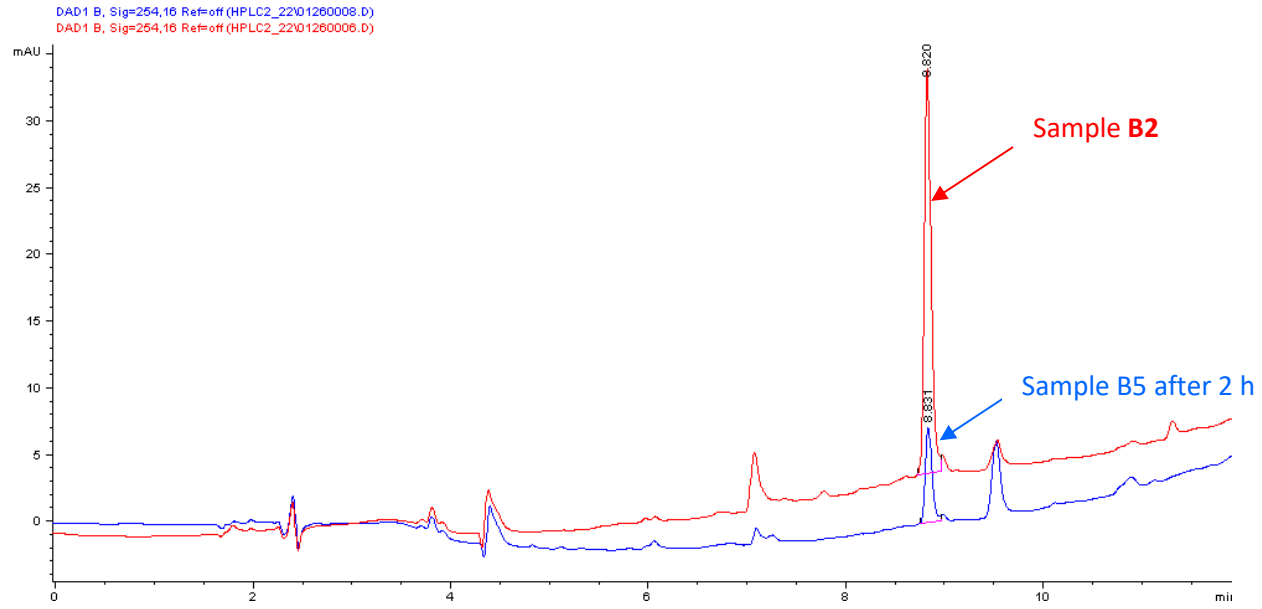
Appendix: Typical Kit Performance Data (LC Analysis, CellMosaic)

Lot#: S309.S10.0127B

Cys-peptide information: Transducin alpha peptide (340-350) containing a Cys in the middle (Cat# CM29202 from CellMosaic). Sequence: IKENLKDCGLF; MW: 1279.5 Da; Total amount added: 5 mg

Maleimide agarose: 1 mL settled beads

Figure 1: Overlay C18 HPLC/UV analysis of Transducin alpha peptide solution before (sample from step **B2,** red trace) and after immobilization (sample **B5** after 2 h reaction, blue trace) (detected at 254 nm).



Summary of the result:

Sample Name	Dilution Factor	HPLC Peak Area at 254 nm	% of Peptide Consumed	Peptide Loading (mg/mL)	Peptide Loading (µmole/mL)
B2	10	148	N/A		
B5 after 2 h	10	34	78%	3.90	3.0