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PerKit™ Streptavidin Oligo Conjugation Kit (Single Label via Click Chemistry) (CM52427) User Reference Guide

Contents

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Important Notes & Contact Information

READ BEFORE USING ANY RESOURCES PROVIDED HEREIN

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

Sample data if provided, is provided solely for illustrative purposes and as examples of a small dataset used to verify kit performance within the CellMosaic laboratory. Information regarding the chemicals and reagents used in the kit is included where necessary.

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Kit Configuration and Components

CellMosaic has designed this PerKit® for single-labeled streptavidin-oligo conjugation using click chemistry. This kit offers multiple configurations to accommodate various oligo lengths and can be used to conjugate 2 to 5 nanomoles (nmol) of oligo. **Table 1** provides the catalog numbers for different kit configurations, and **Table 2** lists the kit components.

Table 1: Configurations of the PerKit™ Streptavidin−Oligo conjugation kit (CM52427)

Configuration	No. of Reactions	Catalog No.
1. Labeling for short oligo (5−30 bases)	1	CM52427.1x1
	3	CM52427.1x3
2. Labeling for medium oligo (≥30 bases)	1	CM52427.2x1
	3	CM52427.2x3

How to use this protocol: The protocol in this user manual is written for two configurations: a 5−30 bases oligo (CM52427.1) and an oligo with ≥30 bases oligo (CM52427.2). The steps are common to both configurations; however, some kit components are specific to each configuration.

⚠ Please follow the specific instructions provided for each configuration.

Requirements for DBCO-oligo:
1. Total amount: 2 to 5 nanomoles of oligo, as measured by UV.
2. Purity: HPLC-purified, with DBCO-oligo content ≥90% as measured by C18 HPLC.
3. Length: Minimum of 5 bases (Note: Oligos longer than 60 bases may lead to lower loading efficiency and increased impurities).

***Note*:** HPLC-purified DBCO-modified oligos, up to 90 bases in length, can be purchased from standard oligo manufacturers for this kit preparation. It is highly recommended that customers analyze and quantify oligos prior to use.

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Table 2: Components and storage temperatures for the PerKit® streptavidin−oligo conjugation kit. All kits share the same components, except for the type of Filter Devices.

- Upon receipt, please remove **Box 1** and store it in a freezer at or below -20°C.
- Store **Box 2** in a refrigerator at 2−8°C.

Box No. (Storage T)	Name	Cat#	Kit Cat#	Quantity (x1)	Quantity (x3)
Box 1 (≤-20°C)	Azide-activated Streptavidin (**red label**)	CM52147	Any	1 unit	3 units
Box 2 (2−8°C)	Solution A (**green label**)	CM01003	Any	4 mL	12 mL
	Buffer A (Equilibrium and Washing Buffer, **orange label**)	CM02036		4 mL	10 mL
	Buffer B (Elution buffer, 50 mM Tris buffer, pH 8.0, 1M NaCl, **navy label**)	CM02004		1.5 mL	5 mL
	Filter Devices (Only one part# will be supplied)	CM03CD003A	CM52427.1	1	3
		CM03CD010A	CM52427.2		
	Collection Tubes for Filter	CM03CT0	Any	2	6
	Column Q	CM03SC5		1	3
	Collection Tubes for Column Q	CM03CT6		2	6
User Supplied Material	DBCO-Oligo (≥5 bases)	N/A		NOT PROVIDED (2−**5 nano-mole)** for each reaction)	

Safety Information

Warning: Some chemicals used in the kit may be hazardous and can cause injury or illness. Please read and understand the Safety Data Sheets (SDS) available at www.CellMosaic.com before storing, handling, or using any materials.

Labeling Chemistry

The kit is designed for use with DBCO-modified oligos, which the user supplies. These oligos are readily available from many commercial oligo suppliers. Using the kit components, the user can react the DBCO-oligo with azide-activated streptavidin in a single step to generate the streptavidin-oligo conjugates. The total length of the linkage between the streptavidin and DBCO-oligo is 16 atoms. The Q-column purification step typically yields streptavidin-oligo conjugate with a purity of greater than 80%.

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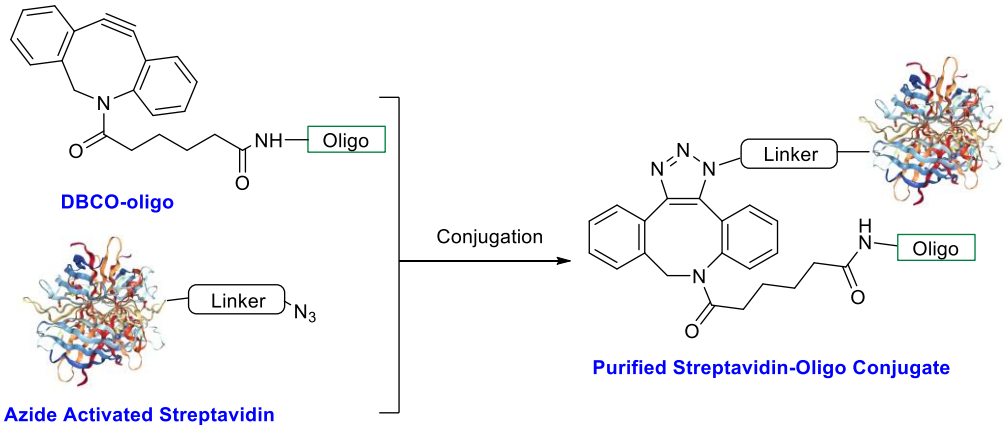

Scheme 1. Synthetic route to streptavidin−oligo conjugate.

An HPLC purified oligo is required for this conjugation. If the DBCO-oligo content is >90%, the final conjugate typically achieves >90% purity, with an average of one oligo per streptavidin (predominantly single-labeled). This purity is sufficient for ELISA, as trace impurities have minimal effect on signal intensity. For oligos longer than 60 bases or modified with non-phosphate backbone or bases, DBCO-oligo purity may be lower. Additionally, solubility issues in aqueous buffer can lead to inefficient labeling, resulting in low loading and high impurities. For complete removal of oligo impurities, gel filtration chromatography is recommended.

Key Features of the Streptavidin-Oligo Conjugation Kit:

- High-quality azide-activated streptavidin for precise single labeling.
- Single purification step yields over 90% streptavidin-oligo conjugates, with the majority being single-labeled, assuming oligo quality meets required standards.
- Quick preparation process, completed in a single day.
- Stable linkage with an optimal spacer to prevent interference between oligo binding and streptavidin activation.
- Included all necessary reagents, from preparation to purification.
- Options for tailored post-conjugation services at CellMosaic:
    - You can send your conjugates to CellMosaic for HPLC analysis, complete removal of trace oligo impurities, or elimination of unreacted Streptavidin.

Support

Customers can request recommendations for conjugation if the oligo has special features or solubility issues. CellMosaic also provides fee-based support services for customers who need assistance with HPLC analysis of final conjugates and further purification to remove trace oligo impurities.

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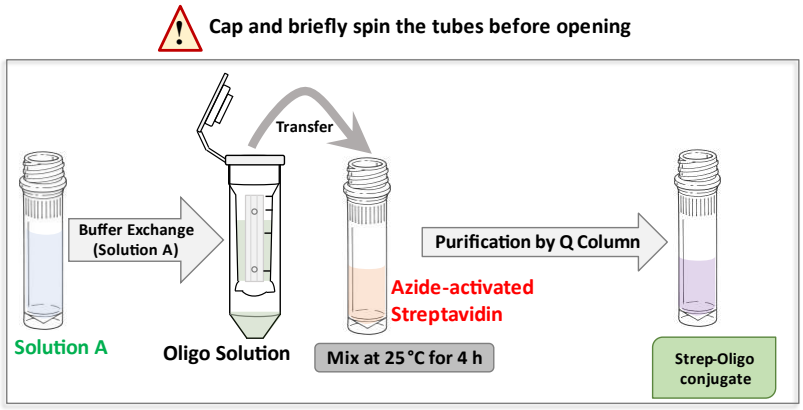
Protocol

This protocol involves a single reaction. Streptavidin-oligo conjugation is typically complete within 4 hours, though it can be extended or left at 2–8°C overnight if needed. The Q-column purification step takes less than 10 minutes.



Scheme 2: Schematic diagram of the workflow for preparing streptavidin–oligo conjugates.

1. Lab Instrumentation Needed

- Vortex mixer, centrifuge (preferably refrigerated, 14,000 g capable), mini-centrifuge
- Pipettes and tips
- Timer
- Incubator or shaker set at 25°C
- Personal protection equipment (lab coat, safety glasses, and chemical-resistant nitrile gloves)
- UV spectrophotometer (optional)

2. Preparation of Oligo Sample for Labeling

Items needed: DBCO-Oligo (user supplied), 1 Filter Device (CM03CD003A or CM03CD010A), 2 Collection Tubes (CM03CT0), Solution A (CM01003, green label), clean centrifuge tubes (not provided in the kit).

The total amount of DBCO-oligo used for the conjugation is **2–5 nanomole**.

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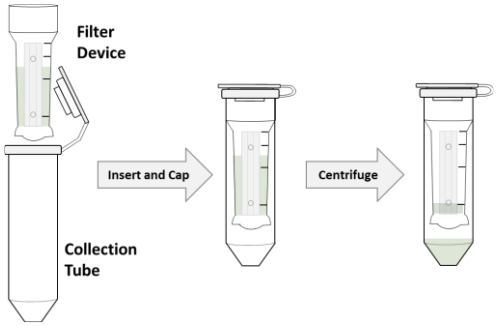
A1. Insert a new Filter Device into one of the provided Collection Tubes. Follow the appropriate step based on the condition of your oligo.

- ✓ **If your oligo is supplied as a lyophilized solid**: Dissolve the oligo in deionized water to a concentration of 50 µM, then transfer up to 100 µL to the Filter Device. Add Solution A (green label) to bring the total volume to 500 µL, then cap it.
- ✓ **If your oligo is supplied as liquid**: Transfer up to **5 nanomoles** directly to the Filter Device. Add Solution A to bring the total volume to 500 µL, then cap it.



A2. Place the capped Filter Device in the centrifuge rotor, positioning the cap strap toward the rotor's center. Use a similar device to counterbalance.

A3. Centrifuge the Filter Device at 14,000 x g (preferably cooled to 4°C) for 8 to 15 minutes to concentrate the solution to < 100 µL. Spin time will vary based on the Filter Device provided in the kit.

Catalog No# (1 rxn, 3 rxn)	Oligo Length	Spin Time	Typical Leftover Vol.
CM52427.1(x1, x3)	5-30 bases	15	80 µL
CM52427.2(x1, x3) CM52427.3(x1, x3)	≥31 bases	8	35 µL
Typical leftover volume is obtained using an Eppendorf 5417R and centrifuge at 4°C.			

A4. Remove the assembled device from the centrifuge and separate the Filter Device from the Collection Tube. Transfer the filtrate from the Collection Tube to a clean centrifuge tube (not provided). **Retain the filtrate until the experiments are complete.**

A5. Reinsert the Filter Device into the collection tube. Add 400 µL of Solution A to the Filter Device, then spin at 14,000 x g for 8 to 15 minutes to concentrate the solution to < 100 µL. Remove the device from the centrifuge. Transfer the filtrate from the Collection Tube to a clean centrifuge tube (not provided). **Retain the filtrate until the experiments are complete.**

3. Conjugation

Items needed: DBCO-Oligo from Step **A5**, Azide Activated Streptavidin (CM52147, red label), Solution A (CM01003, green label).

B1. Briefly centrifuge the tube containing azide-activated streptavidin (red label).

B2. Add 125 µL of Solution A (green label) to the azide-activated streptavidin tube. Vortex for 30 seconds to 1 minute to fully dissolve the streptavidin.

B3. Transfer the DBCO-oligo sample from the Filter Device (from **Step A5)** to the azide-activated streptavidin tube by pipetting, preferably using a sterilized 100 µL pipette tip with a filter. Ensure the pipette tip reaches the bottom of the Filter Device.

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B4. Wash the Filter Device twice with 20 µL of Solution A, transferring each wash to the azide-activated streptavidin tube from **Step B3 (*Note:* For each wash, add buffer, then aspirate with the pipette 2-3 times).**

B5. Incubate at 25°C (or room temperature) for 2-4 hours. For oligos longer than 31 bases, a longer incubation time is acceptable, including overnight.

Start Time: _____ End Time: _____

4. Purification to Remove Excess Streptavidin

Items needed: Streptavidin-Oligo Reaction Mixture from Step **B5**, Buffer A (Equilibrium and Washing Buffer, CM02036, orange label), 1 Column Q (CM03SC5), Buffer B (Elution Buffer, CM02004, navy label), 2 Collection Tubes (CM03CT6).

C1. Sample Preparation: When **Step B5** is complete, add 175 µL of Buffer A (orange label). Mix by pipetting up and down three times.

C2. Column Equilibration: Insert Column Q into one of the provided Collection Tubes. Add 400 µL of Buffer A to a Column Q, then centrifuge at 2000 x g for 2 minutes. Discard the flow-through. Repeat this step once more.

C3. Sample Application: Add the sample solution from **Step C1** to the equilibrated Column Q, up to a maximum volume of 400 µL. Centrifuge at 2000 x g for 5 minutes. Set aside the flow-through.

C4. Washing: Add 400 µL of Buffer A to Column Q and centrifuge at 2000 x g for 2 minutes. Repeat this washing step two more times. Discard the flow-through.

C5. Elution: Place Column Q onto a clean Collection Tube. Add 400 µL of Buffer B (navy label) to the column, then centrifuge at 2000 x g for 2 minutes. Collect the flow-through and label it as Elution 1.

> ***Tip for elution volume:*** **Elution 1** contains most of your purified streptavidin-oligo conjugate. However, when conjugating longer oligos, a larger volume may be required for complete elution. Repeat **Step C5** to collect additional fractions if needed.

Streptavidin-Oligo is Ready for Your Experiment

The approximate concentration of **Elution 1** is 6.25 µM in 400 µL of 50 mM Tris buffer, pH 8.0 with 1 M NaCl, assuming a 50% recovery from 5 nmol oligo-scale reaction. Elution can be diluted for a lower concentration, or a desalting column (not included in kit) can be used for buffer exchange.

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Other Considerations

1. Concentration Determination for Oligo (Unlabeled)

Accurately measuring the amount of oligo is essential for optimized labeling in this protocol. Oligo manufacturers typically supply the oligo in lyophilized form, with the amount quantified prior to lyophilization, which is generally sufficient. For precision, you may re-measure the concentration of the oligo after re-suspending it in deionized water in **Step A1**.

$$Concentration\ (M)\ of\ oligo\ before\ dilution = \frac{(A260) \times DF}{L \times \varepsilon o}$$

A260: UV absorbance of the oligo at 260 nm.

L: UV cell path length (cm) - if using a 1 cm UV cell, dilute the oligo in water to a concentration of 1 to 2 µM.

DF: Dilution Factor.

Ɛo: Extinction coefficient of the oligo.

2. Concentration Determination for Streptavidin–Oligo Conjugate

To determine the concentration of your conjugate, dilute the streptavidin–oligo from **Step C5** with 1x PBS buffer. Measure the UV absorbance of the streptavidin–oligo at 260 nm (A_{260}) using a UV spectrometer, then calculate the concentration using the following formula:

$$Concentration\ (\mu M)\ of\ the\ diluted\ sample = \frac{\mathrm{A260} \times 1000000}{\mathrm{L} \times (\varepsilon \mathrm{o} + 84000)}$$

A260: UV absorbance of the conjugate at 260 nm.

L: UV cell path length (cm) - if using a 1 cm UV cell, dilute conjugate 4 to 8 times to obtain an accurate reading

Ɛo: Extinction coefficient of oligo at 260 nm.

3. MW Calculation

Calculation of the MW of the 1:1 conjugate:

Mw(conjugate) = Mw(oligo) + 52000

4. Recommended Storage Conditions

The recommended storage is 2-8°C. For long-term storage, you may either freeze the sample below -20 °C or lyophilize it.

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5. Characterization of Streptavidin–Oligo by HPLC

Streptavidin–oligo conjugate can be characterized using size-exclusion chromatography (SEC) and anion exchange chromatography (AEX) HPLC.

- **SEC:** This method separates molecules based on apparent molecular weight (MW) or size in aqueous solution. Larger MW conjugates elute earlier. By comparing the SEC profiles of unlabeled streptavidin, unlabeled oligo, and the conjugate, you can determine whether conjugation occurred, assess labeling heterogeneity, and estimate the percentage of unlabeled oligo and streptavidin.
- **AEX**: This technique separates molecules based on net surface charge. After conjugation, the total negative charge of the conjugates may change, allowing separation. However, obtaining consistent data with AEX HPLC may require optimization.

We recommend using SEC as the primary method for conjugate analysis. CellMosaic offers two SEC standards (Product #: CM92004 and CM92005) for use with any SEC column. The CM92004 product sheet provides detailed information and methodology for running SEC HPLC analysis.

If you do not have access to an HPLC facility, you may send your sample to CellMosaic for analysis.

6. Sample Submission for HPLC Analysis

If you are submitting samples to CellMosaic for HPLC analysis, please follow these instructions:

1) **Order**: Go to https://www.cellmosaic.com/hplc-analysis/, select SEC HPLC Analysis (Product# AS0023) and/or AEX HPLC Analysis (Product# AS0026), choose the quantity (bulk discounts are available for multiple samples), and submit the order. Alternatively, you can email info@cellmosaic.com for a quote and to place the order.
2) **Prepare Unconjugated Oligo Sample**: Dilute the un-conjugated oligo to 10-20 µM in PBS buffer, then transfer 50 µL of the diluted solution to a 0.5 mL micro-centrifuge tube. Label the tube clearly.
3) **Prepare Conjugate Sample:** Dilute the conjugate to 0.1–0.5 mg/mL in PBS buffer, then transfer 50 µL of the diluted solution to a 0.5 mL micro-centrifuge tube. Label the tube clearly.
4) **Shipping:** Ship your samples with a cold pack for overnight delivery.

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

Oligo information: HPLC-purified 23mer oligo with 5' DBCO modification
Kit Lot number: Box 1 (S553.S8.1104D) and Box 2 (S553.S7.1104)

Figure 1: Overlay of Size-exclusion HPLC profiles of streptavidin (green trace), oligo (red trace), and purified product (**Step C5, Elution1**) (blue trace) detected at 220 nm. SEC separates conjugates based on apparent molecular weight (MW) or size in aqueous solution, with larger MW eluting earlier.

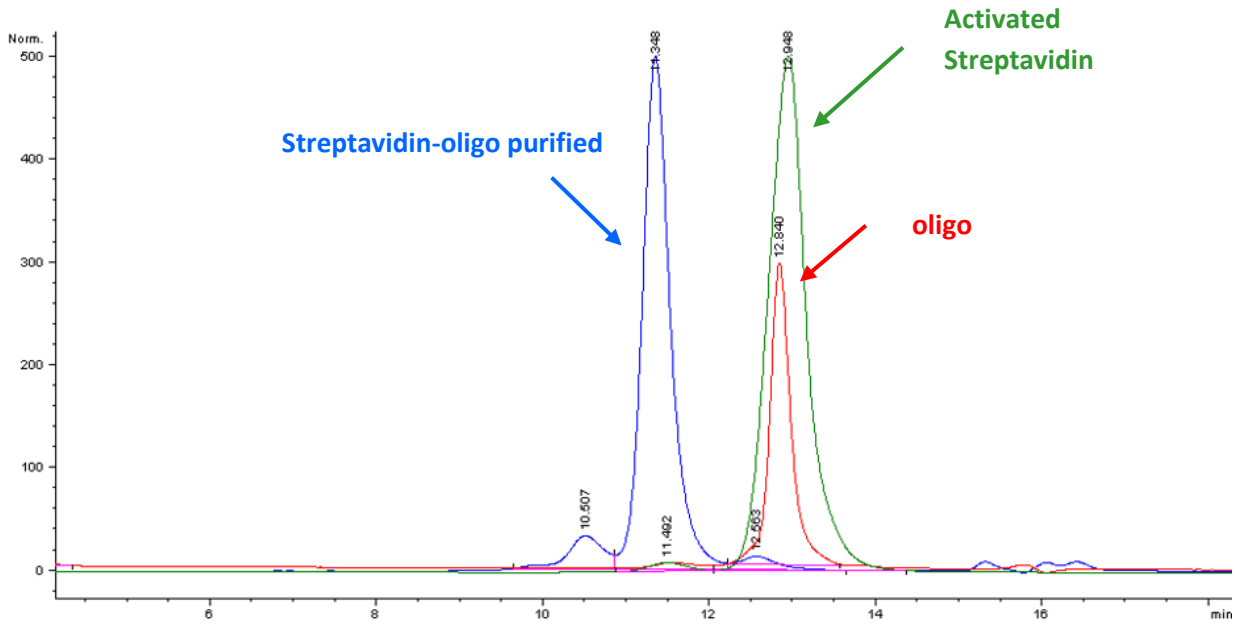

Figure 2: SEC HPLC of purified streptavidin-oligo conjugate detected at 220 nm (**Step C5, Elution1**).

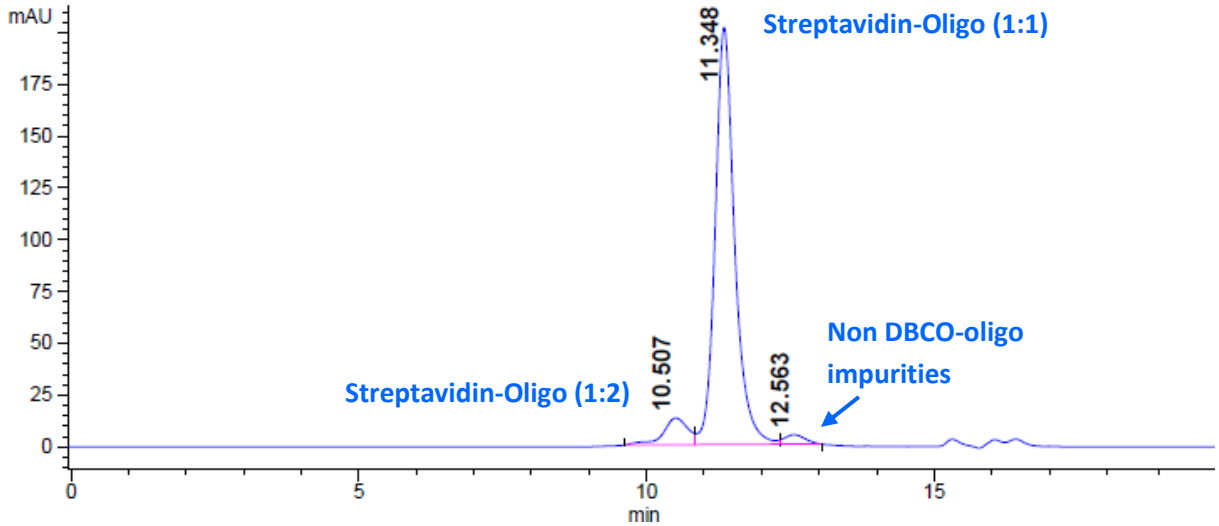

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Table 3: Summary of Results

Sample	Volume (μL)	% free Streptavidin	% free oligo (non-DBCO impurities)	% of Streptavidin/Oligo Conjugates
E1 (first elution)	400	Not detected	2	97.9% (90.5% of 1:1 conjugates)